



Acutrade Animal Feed Probiotic ***Product Information Sheet***

Acutrade Animal Feed Probiotic are becoming increasingly relevant in the poultry industry, due to the phasing out of antibiotic growth promoters (AGP) worldwide. Probiotics are "live microbes that, when administered in adequate amounts, confer a health benefit to the host". Probiotics support a healthier digestive tract, due to varying modes of action, such as improving digestion, regulating intestinal microflora, improving gut barrier function, preventing gastrointestinal diseases, and modulating the immune system. The genus *Bacillus* confers several advantages such as survival in the harsh feed manufacturing processes, longer shelf life and robustness to the fluctuating conditions within the chicken gastrointestinal tract.

It has been shown that **Acutrade Animal Feed Probiotic** resulted in improved broiler growth rate and feed utilization efficiency. They also enhance immune response confer protection against pathogens, colonize the GIT of chickens and improve the histomorphology of intestinal villi. These effects endorse research into their use as natural alternatives to AGP products. The ban on the use of AGPs in poultry production has challenged the industry need to prevent poultry diseases. Although zoonotic diseases, such as salmonellosis and listeriosis, have negative effects on the industry, losses in broiler production is most severe due to *Clostridium perfringens* infections, which causes acute necrotic enteritis (NE), resulting in production losses correlated an increased incidence of NE with the banning of AGPs. This pathogen also causes sub-clinical necrotic enteritis (SNE), presenting as necrotic dermatitis, cholangiohepatitis, gizzard erosion, and lower production efficiency.



When developing probiotics, the use of microorganisms indigenous to poultry is preferred, as it not only gives the best chance of probiotic survival and colonization of the GIT but also alleviates many of the challenges associated with the inclusion of foreign bacteria. This study evaluates the in-vivo efficacy of a novel multi-strain probiotic comprising of **Acutrade Animal Feed Probiotic** on growth performance and intestinal histomorphological parameters of broilers fed diets containing 2 ME levels. The broilers were exposed to *C. perfringens* at sub-clinical levels to challenge the birds such that the probiotic effect could be thoroughly evaluated.

A broiler performance analysis with **Acutrade Animal Feed Probiotic** was conducted with the following results achieved:

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Table 3. Growth performance of broilers fed diets containing either a standard (100%) or reduced (98%) metabolizable energy level with or without *Bacillus* spp. based probiotics.

	Standard ME			Reduced ME			SEM	P value		Energy × probiotic treatment
	Without probiotics	Commercial probiotic	Test probiotic	Without probiotics	Commercial probiotic	Test probiotic		Probiotic treatment	ME	
Body weight (g)										
Day 1	40.87	40.69	40.5	41.05	41.05	40.65	0.23	0.242	0.233	0.883
Day 7	177.8	178.9	179.5	177.6	177.1	177.4	1.75	0.904	0.340	0.844
Day 14	448.5	451.3	450.7	443.1	442.6	446.8	5.04	0.845	0.152	0.885
Day 21	827.4	820.5	821.5	818.1	803.1	817.4	9.54	0.635	0.122	0.756
Day 28	1,366 ^{a,b}	1,363 ^{a,b}	1,388 ^a	1,349 ^{a,b}	1,324 ^b	1,367 ^{a,b}	18.01	0.170	0.087	0.808
Day 35	1,906 ^{a,b}	1,940 ^{a,b}	1,957 ^{a,b}	1,881 ^b	1,892 ^{a,b}	1,974 ^a	30.66	0.065	0.470	0.564
Feed intake (g/bird)										
Day 0-7	174.4	176.5	172.7	177.7	180.6	174.9	2.78	0.239	0.167	0.941
Day 0 to 14	522.1	520.2	514.3	527.5	530.3	526.1	6.46	0.963	0.488	0.771
Day 0 to 21	1,222 ^a	1,164 ^b	1,160 ^b	1,116 ^b	1,126 ^b	1,121 ^b	19.7	0.166	0.001	0.312
Day 0 to 28	2,218 ^a	2,163 ^a	2,181 ^a	2,021 ^b	2,026 ^b	2,050 ^b	30.5	0.682	<0.0001	0.505
Day 0 to 35	3,203 ^a	3,144 ^{a,c}	3,176 ^{a,d}	2,992 ^b	2,977 ^b	3,065 ^{b-d}	43.9	0.393	<0.0001	0.526
Feed conversion ratio (g: g)										
Day 0 to 7	1.27 ^{a,b}	1.28 ^{a,b,c}	1.22 ^b	1.30 ^{a,c}	1.33 ^c	1.28 ^{a,c}	0.02	0.023	0.005	0.732
Day 0 to 14	1.58 ^{a,b,c}	1.53 ^{a,b}	1.50 ^a	1.59 ^{b,c}	1.62 ^c	1.58 ^{a,b,c}	0.03	0.757	0.043	0.599
Day 0 to 21	1.72 ^b	1.62 ^a	1.63 ^a	1.60 ^a	1.61 ^a	1.58 ^a	0.03	0.074	0.137	0.120
Day 0 to 28	1.78 ^d	1.72 ^{c,d}	1.69 ^{b,c}	1.64 ^{a,b}	1.65 ^{a,b}	1.63 ^a	0.02	0.142	<0.0001	0.099
Day 0 to 35	1.78 ^c	1.69 ^b	1.68 ^b	1.69 ^b	1.68 ^{a,b}	1.61 ^a	0.02	0.002	0.0003	0.263

^{a-d}Values without a common superscript in the same row differ significantly ($P < 0.05$, $n = 12$).

Within the treatment groups that received the reduced ME diets, the test probiotic treatment resulted in a significantly higher final BW (day 35) compared to the negative control ($P = 0.03$) and was 4.1% higher than the commercial probiotic, but this difference was not significant (Table 3). FCR within the reduced ME diets were positively influenced by supplementation of the test probiotic from day 28 onwards, resulting in significantly better FCR than the negative control ($P < 0.05$). The FCR of the test probiotic was also 4.3% better than the commercial probiotic but the difference was not significant.

The benefit of **Acutrade Animal Feed Probiotic** supplementation on broilers fed with the reduced ME diet is illustrated in Figure 1. In all of the responses (BW, FI, and FCR), the test probiotic supplementation resulted in improved performance than the un-supplemented and commercial probiotic treatment groups, but was only significantly better than the un-supplemented group for final BW and FCR.

The total mortality rate for all 6 experimental groups was only 3.7% for the duration of the trial and no effect of treatment could be correlated to mortality.

A significant effect of **Acutrade Animal Feed Probiotic** treatment was noted for FCR at day 35 (Table 3). Irrespective of probiotic supplementation, a suppressing effect on FI from day 21 onwards was observed, when broilers were fed a reduced ME diet in comparison to the standard ME diet ($P < 0.0001$). No significant interaction effects between ME level and probiotic treatment were observed.

Lesion Scoring and Liver Weight

In broilers fed the standard ME diets, the cumulative lesion scores across the 3 regions of the small intestine (duodenum, jejunum, and ileum) were 22, 17, and 19 for the un-supplemented control, the commercial probiotic and the test probiotic treatments, respectively. Similarly, in the reduced ME diets the cumulative lesion counts were 32, 12, and 26, respectively. No significant differences were found in the numbers and severity of lesions between any of the treatment groups.

For both levels of dietary ME, the livers of broilers that received the **AcuTrade Animal Feed Probiotic** weighed significantly less than those of both the commercial probiotic and un-supplemented groups (Table 4). Irrespective of probiotic treatment, the ME content of the diets had a highly significant ($P < 0.0001$) effect on the liver weight of broilers when expressed as a percentage of BW. The livers of broilers that received the reduced ME diets were heavier than those fed the standard ME diet.

Histomorphology of the Gastrointestinal Tract

In broilers fed the standard ME diet, there was no significant difference in villi height or crypt depth of the small intestine between any of the treatments (Table 4). However, broilers fed the standard ME diet supplemented with the commercial probiotic resulted in significantly higher VCR ($\mu\text{M} : \mu\text{M}$) within the duodenum and ileum when compared to the negative control. Similarly, the **AcuTrade Animal Feed Probiotic** treatment also resulted in higher ratios, but the differences were not significant.

Broilers fed the reduced ME diets containing the **AcuTrade Animal Feed Probiotic**, displayed significantly longer duodenal villi when compared to both the un-supplemented ($P = 0.01$) and the commercial probiotic ($P = 0.03$) groups (Figure 2). Both duodenal and jejunal crypt depths of broilers fed either of the probiotics supplemented in the reduced ME diets were significantly shallower than in the un-supplemented treatment group. Additionally, the VCR in both the duodenum and the jejunum were significantly higher for the broilers that received either one of the probiotic treatments under reduced ME conditions (Table 4 and Figure 2).

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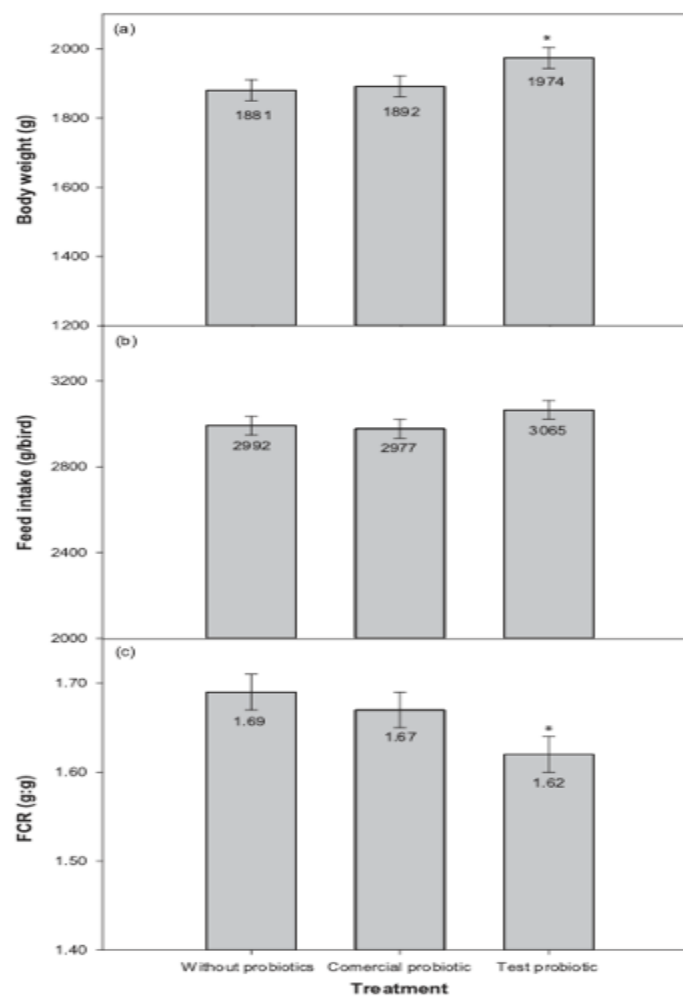


Figure 1. The broiler performance of birds fed the reduced ME diet supplemented with or without probiotics. a) The body weights of birds at day 35. b) The cumulative feed intake from day 0 to 35. c) The cumulative mortality adjusted FCR from day 0 to 35.* are represented as significant difference ($P < 0.05$) over the control.

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Table 4. Intestinal villi histomorphology and liver weight of broilers fed diets containing either a standard (100%) or reduced (98%) metabolizable energy level with or without *Bacillus* spp. based probiotics.

	Standard ME			Reduced ME			SEM	P value		
	Without probiotics	Commercial probiotic	Test probiotic	Without probiotics	Commercial probiotic	Test probiotic		Probiotic treatment	ME	Energy × probiotic treatment
Duodenum										
Villi height (μM)	1,634 ^b	1,834 ^{a,b}	1,807 ^{b,c}	1,664 ^b	1,711 ^b	1,936 ^{a,c}	73.9	0.015	0.844	0.238
Crypt depth (μM)	195.3 ^b	184.0 ^{b,c}	200.2 ^b	252.6 ^a	165.4 ^c	201.8 ^b	9.43	<0.0001	0.088	0.0006
Villi: crypt ($\mu\text{M} : \mu\text{M}$)	8.430 ^c	10.24 ^{a,d}	9.138 ^{c,d}	6.713 ^b	10.52 ^a	9.763 ^{a,c}	0.49	<0.0001	0.499	0.042
Jejunum										
Villi height (μM)	1,184 ^{a,b}	1,166 ^{a,b}	1,275 ^a	1,093 ^b	1,179 ^{a,b}	1,243 ^{a,b}	59.7	0.126	0.459	0.684
Crypt depth	174.1 ^b	182.9 ^b	195.5 ^{a,b}	219.0 ^a	133.5 ^c	171.1 ^b	11.9	0.007	0.325	0.0006
Villi: crypt ($\mu\text{M} : \mu\text{M}$)	6.853 ^a	6.883 ^a	6.524 ^a	5.108 ^b	8.925 ^c	7.477 ^a	0.45	0.0003	0.258	0.0003
Ileum										
Villi height (μM)	775.3 ^{a,b}	801.1 ^{a,b}	872.2 ^a	758.5 ^b	818.3 ^{a,b}	857.0 ^{a,b}	39.4	0.054	0.880	0.889
Crypt depth (μM)	176.8	151.9	179.1	195.9	158.2	190.1	17.6	0.144	0.401	0.936
Villi: crypt ($\mu\text{M} : \mu\text{M}$)	4.437 ^{b,c}	5.493 ^{a,d}	4.953 ^{c,d}	3.915 ^{b,c}	6.133 ^a	4.895 ^{b,d}	0.36	0.0002	0.946	0.281
Liver weight (%)	2.32 ^b	2.44 ^{b,d}	2.06 ^c	2.61 ^{a,d}	2.68 ^a	2.35 ^b	0.06	<0.0001	<0.0001	0.930

^{a-d}Values without a common superscript in the same row differ significantly ($P < 0.05$).

Data represents means based on 12 replicates per treatment.

Villi: crypt = villi height to crypt depth ratio.

Liver weight expressed as percentage of body weight.

CONCLUSIONS

The multi-strain *Bacillus* **AcuTrade Animal Feed Probiotic** product improved growth performance and generally had a positive effect on *C. perfringens* challenged-broiler well-being, indicated by gut and liver health observations. Furthermore, the **AcuTrade Animal Feed Probiotic** effect appeared to be better than the commercial probiotic product. Some of the key driving factors for industry adoption include improved performance in cheaper reduced ME diets and better resistance to *C. perfringens* challenge. Our results further substantiate the attractiveness of multi-strain *Bacillus* **AcuTrade Animal Feed Probiotic** as a replacement to other undesirable in-feed growth promoting and antibacterial additives. Future considerations include testing the novel probiotic under commercial conditions, as well as elucidating the modes of action of its functionality.

STORAGE AND DOSE INSTRUCTIONS

Storage: Store in cool ventilated area

Application & Concentration: Normal Feeds add 0.1% up to 0.5%

Speciality Feeds add 0.5% up to 2%

Contact Time: N/A

PH levels – Undiluted (PH 9.5 – 10)

Toxicity: Toxicological information / health effects

Inhalation – Wear Dust Mask.

Skin Contact - None.

Eye Contact – Will cause irritation, seek medical assistance.

Ingestion – None Known.

Biodegradability - Biodegradable ingredients.

Eco Toxicity - Non-toxic.

Specific Effects - None experienced or reported. Irritant to eye.

Safety Precautions – Wear appropriate safety gear i.e. Safety glasses & respiratory mask.

