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Evaluation of MANNANASE VTR in broilers

REPORT N° I10412017-3

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1. BACKGROUND

The evaluation was conducted by LIAN Desarrollo y Servicio S.A.C. implementing the proposal P10582016-4 sent to Protecno Peru S.A.C.

2. OBJECTIVES

The aims of the present study were to:

- Determine the effectiveness of the metabolizable energy matrix of the MANNANASE VTR.
- Determine the effect of adding this additive in the diet without applying an energy matrix (on top) on performance.
- Compare both feeding strategies: on top versus applying a matrix.

3. MATERIALS AND METHODS

Place: The evaluation was conducted by LIAN Desarrollo y Servicio S.A.C. within La Molina National Agrarian University facilities, in Lima - Peru, in summer time.

Experimental birds and facilities: 280 day-old Hubbard Classic M77 male broiler chicks were neck-tagged (Figure 1) and randomly assigned to 40 Petersime battery cages with five levels and 20 cages each (Figure 2) until 21 days of age at a ratio of seven chicks per cage. They were then transferred to grower cages (Figure 3) until the end of the evaluation (42 days of age). Chicks were kept assigned to the same experimental unit throughout the evaluation.

Figure 1. Neck-tagging system applied in the evaluation.

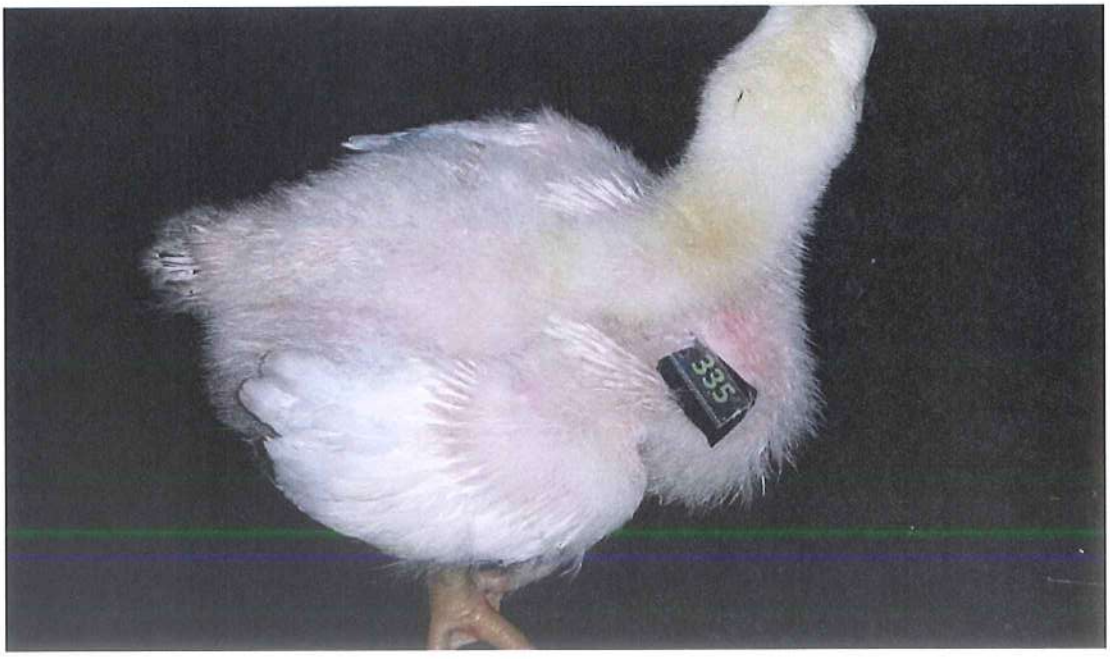

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Figure 2. Cages used for the evaluation from 1 to 21 days of age.

Figure 3. Cages use for the evaluation from 22 to 42 days of age.

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Treatments: The characteristics of experimental treatments are shown in Table 1. Experimental diets were prepared applying procedures designed to ensure that the only differences among them were the adding of the evaluated product and the metabolizable energy content. Treatment were randomly assignment to experimental units. In the first phase the four treatments were also randomly assigned among the four cages within each battery level.

Table 1. Treatments applied in the evaluation.

Treatment	Code [1]	Description
T1	D	Positive control (standard diet).
T2	A	Negative control (same to T1 but with 100 kcal/kg less metabolizable energy).
T3	B	Same to T1 but with 150 g/t *Mannanase VTR* added to the mix (on top feeding). [2]
T4	C	Same to T2 but with 150 g/t *Mannanase VTR* added to the mix (enzyme with matrix).

[1] Codes used as part of blind test protocol.

[2] *MANNANASE VTR* from GUANGDONG VTR BIO-TECH CO., LTD. Batch: 20160818101. Expiration date 17.08.17. Product provided by the client.

Feeding: During the first three days of age, feed was provided on paper on the floor, and water in fountain type drinkers. Since then water and feed troughs were used. Pellet feed (80°C) was provided *ad libitum* under a three-phase feeding program: Pre-starter from 1 to 10 days (Table 2; crumble), Starter from 11 to 21 days (Table 3; 3 mm diameter) and Growing from 22 to 42 days (Table 4; 3 mm diameter). Diets were formulated according to the genetic line guidelines and nutritional requirements for each phase were determined by a projection of the recommended levels.

Blind test: A blind test protocol was applied during the evaluation that included all critical activities such as treatment assignment to experimental units, feeding the birds and the carried out controls.

Response variables: Quality of chicks was evaluated and initial body weights were recorded to ensure homogeneity and discard initial differences among treatments and experimental units with outlier values (Table 5). Throughout the evaluation performance variables were controlled (Table 6) and toward the end of the study indicators of intestinal content fermentation were also evaluated (Table 7). Thus, in the last week the quality of feces was determined and an indirect clinical evaluation of dysbacteriosis was performed (Panneman and Van Der Stroom-Kruyswijk, 2002; Martínez and Vílchez, 2016); and at the end of the study three chickens per experimental unit were sampled to determine intestinal and intestinal content relative weight.

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Table 2. Composition and nutritional content of Pre-starter diets (0 to 10 days).

Ingredients, %	Treatments [1]			
	T1 (D)	T2 (A)	T3 (B)	T4 (C)
Ground yellow corn	52.17	54.51	52.17	54.51
Soybean meal	39.23	38.78	39.23	38.78
Vegetal oil	4.492	2.593	4.492	2.593
Dicalcium phosphate	1.936	1.932	1.936	1.932
Calcium carbonate	0.956	0.960	0.956	0.960
Salt	0.415	0.414	0.415	0.414
DL-Methionine	0.257	0.254	0.257	0.254
Vitamin and trace mineral premix	0.120	0.120	0.120	0.120
Antifungal	0.100	0.100	0.100	0.100
Choline chloride 60%	0.100	0.100	0.100	0.100
L-Lysine HCL	0.080	0.090	0.080	0.090
Growth promoter	0.050	0.050	0.050	0.050
Mycotoxin binder	0.050	0.050	0.050	0.050
Antioxidant	0.025	0.025	0.025	0.025
L-Threonine	0.006	0.008	0.006	0.008
Mannanase VTR	-	-	0.015	0.015
Inert material	0.015	0.015	-	-
Total	100.0	100.0	100.0	100.0
Nutrient	**Calculated content**			
Metabolizable energy, kcal/kg [2]	3,000	2,900	3,000	2,900
Crude protein, %	23.000	23.000	23.000	23.000
Crude fiber, %	3.0417	3.0677	3.0417	3.0677
Calcium, %	1.0000	1.0000	1.0000	1.0000
Available phosphorus, %	0.5000	0.5000	0.5000	0.5000
Sodium, %	0.1800	1.8000	0.1800	1.8000
Chloride, %	0.3043	0.3064	0.3043	0.3064
Electrolyte balance, mEq/kg	247.24	246.74	247.24	246.74
Digestible arginine, %	1.4509	1.4490	1.4509	1.4490
Digestible lysine, %	1.2300	0.1200	1.2300	0.1200
Digestible methionine, %	0.5839	0.5827	0.5839	0.5827
Digestible Met + Cis, %	0.9000	0.9000	0.9000	0.9000
Digestible threonine, %	0.7800	0.7800	0.7800	0.7800
Digestible tryptophan, %	0.2461	0.2450	0.2461	0.2450
Digestible valine, %	1.0670	1.0656	1.0670	1.0656
Linoleic acid, %	3.7082	2.7271	3.7082	2.7271

[1] Codes used as part of the blind test protocol are indicated in brackets.

[2] Does not include the metabolizable energy supplied by the evaluated additive inclusion.

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Table 3. Composition and nutritional content of Starter diets (11 to 21 days).

Ingredients, %	Treatments [1]			
	T1 (D)	T2 (A)	T3 (B)	T4 (C)
Ground yellow corn	56.34	58.68	56.34	58.68
Soybean meal	34.27	33.82	34.27	33.82
Vegetal oil	5.360	3.461	5.360	3.461
Dicalcium phosphate	1.716	1.713	1.716	1.713
Calcium carbonate	1.142	1.146	1.142	1.146
Salt	0.415	0.414	0.415	0.414
DL-Methionine	0.252	0.250	0.252	0.250
Vitamin and trace mineral premix	0.120	0.120	0.120	0.120
Antifungal	0.100	0.100	0.100	0.100
Choline chloride 60%	0.100	0.100	0.100	0.100
L-Lysine HCL	0.022	0.031	0.022	0.031
Growth promoter	0.050	0.050	0.050	0.050
Mycotoxin binder	0.050	0.050	0.050	0.050
Antioxidant	0.025	0.025	0.025	0.025
L-Threonine	0.017	0.018	0.017	0.018
Mannanase VTR	-	-	0.015	0.015
Inert material	0.015	0.015	-	-
Total	100.0	100.0	100.0	100.0
Nutrient	**Calculated content**			
Metabolizable energy, kcal/kg [2]	3,100	3,000	3,100	3,000
Crude protein, %	21.000	21.000	21.000	21.000
Crude fiber, %	2.8803	2.9062	2.8803	2.9062
Calcium, %	1.0000	1.0000	1.0000	1.0000
Available phosphorus, %	0.4500	0.4500	0.4500	0.4500
Sodium, %	0.1800	0.1800	0.1800	0.1800
Chloride, %	0.2935	0.2956	0.2935	0.2956
Electrolyte balance, mEq/kg	224.62	224.12	224.62	224.12
Digestible arginine, %	1.3065	1.3006	1.3065	1.3006
Digestible lysine, %	1.0600	1.0600	1.0600	1.0600
Digestible methionine, %	0.5561	0.5548	0.5561	0.5548
Digestible Met + Cis, %	0.8500	0.8500	0.8500	0.8500
Digestible threonine, %	0.7200	0.7200	0.7200	0.7200
Digestible tryptophan, %	0.2211	0.2200	0.2211	0.2200
Digestible valine, %	0.9719	0.9705	0.9719	0.9705
Linoleic acid, %	4.2249	3.2438	4.2249	3.2438

[1] Codes used as part of the blind test protocol are indicated in brackets.

[2] Does not include the metabolizable energy supplied by the evaluated additive inclusion.

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Table 4. Composition and nutritional content of Grower diets (22 to 42 days).

Ingredients, %	Treatments [1]			
	T1 (D)	T2 (A)	T3 (B)	T4 (C)
Ground yellow corn	61.12	63.46	61.12	63.46
Soybean meal	29.13	28.68	29.13	28.68
Vegetal oil	6.050	4.151	6.050	4.151
Dicalcium phosphate	1.497	1.494	1.497	1.494
Calcium carbonate	1.066	1.070	1.066	1.070
Salt	0.415	0.414	0.415	0.414
DL-Methionine	0.208	0.206	0.208	0.206
Vitamin and trace mineral premix	0.120	0.120	0.120	0.120
Antifungal	0.100	0.100	0.100	0.100
Choline chloride 60%	0.100	0.100	0.100	0.100
L-Lysine HCL	0.033	0.042	0.033	0.042
Growth promoter	0.050	0.050	0.050	0.050
Mycotoxin binder	0.050	0.050	0.050	0.050
Antioxidant	0.025	0.025	0.025	0.025
L-Threonine	0.019	0.020	0.019	0.020
Mannanase VTR	-	-	0.015	0.015
Inert material	0.015	0.015	-	-
Total	100.0	100.0	100.0	100.0
Nutrient	**Calculated content**			
Metabolizable energy, kcal/kg [2]	3,200	3,100	3,200	3,100
Crude protein, %	19.000	19.000	19.000	19.000
Crude fiber, %	2.7221	2.7481	2.7221	2.7481
Calcium, %	0.9000	0.9000	0.9000	0.9000
Available phosphorus, %	0.4000	0.4000	0.4000	0.4000
Sodium, %	0.1800	0.1800	0.1800	0.1800
Chloride, %	0.2960	0.2981	0.2960	0.2981
Electrolyte balance, mEq/kg	201.51	201.01	201.51	201.01
Digestible arginine, %	1.1584	1.1534	1.1584	1.1534
Digestible lysine, %	0.9400	0.9400	0.9400	0.9400
Digestible methionine, %	0.4883	0.4870	0.4883	0.4870
Digestible Met + Cis, %	0.7600	0.7600	0.7600	0.7600
Digestible threonine, %	0.6500	0.6500	0.6500	0.6500
Digestible tryptophan, %	0.1955	0.1943	0.1955	0.1943
Digestible valine, %	0.8748	0.8734	0.8748	0.8734
Linoleic acid, %	4.6559	3.6748	4.6559	3.6748

[1] Codes used as part of the blind test protocol are indicated in brackets.

[2] Does not include the metabolizable energy supplied by the evaluated additive inclusion.

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Table 5. Variables controlled to verify the initial homogeneity.

Variable	Method applied
Maximum body weight, g	The maximum weight found among the seven chickens obtained after weighing each bird individually.
Minimum body weight, g	The minimum weight found among the seven chickens obtained after weighing each bird individually.
Range of weights, g	Difference between the maximum and minimum weights within the cage.
Body weight, g	Average body weight obtained after individually weighing the seven chicks of the cage.
Body weight standard deviation, g	Variation (positive or negative) in the body weight after weighing individually the birds within the cage.
Body weight variation coefficient, %	Percentage body weight variation observed within the experimental unit after weighing individually the birds.

Table 6. Performance variables used in the evaluation.

Variable	Method applied
Body weight, g	Weight obtained by averaging the individual body weights of the chickens in each cage. It was measured weekly.
Body weight gain, g/bird	Calculated with the initial average weight and the average weights obtained at the end of each week.
Body weight gain, g/bird /d	Calculated as the cumulative gain of average weight per day at the end of each week.
Feed intake, g/bird	Cumulative values calculated weekly with: (1) daily feed supply, (2) feed residue when found a dead bird, (3) feed residue at the end of the period, and (4) number of live birds.
Mortality, %	Calculated weekly with the information of the mortality registry (cause, feed residue and weight of the bird).
Commercial feed conversion ratio	Calculated by dividing the total feed intake by the total body weight obtained at the end of the same period (weight of the dead birds are not included).
Corrected by mortality feed conversion ratio	Calculated by dividing the total feed intake by the total body weight obtained at the end of the same period, including the weight of the dead birds.
European Efficiency Index (IEE)	$\text{IEE} = \frac{100 \times \text{average body weight (kg)} \times \text{survival (\%)}}{\text{corrected feed conversion ratio} \times \text{period (days)}}$

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Table 7. Indicators of intestinal content fermentation.

Variable	Method applied
Watery feces, %	Feces were collected on absorbent paper during three days in the last week of evaluation. The excreta was considered wet if the diameter of humidity was at least twice the one of the dropping (Panneman and Van Der Stroom-Kruyswijk, 2002). Averages were obtained per experimental unit.
Feces with undigested feed, %	Sampled feces were inspected and the presence of undigested feed was determined.
Feces with desquamated mucosa, %	Sampled feces were inspected and the presence or absence of desquamated mucosa was determined.
Bloody feces, %	Sampled feces were inspected and the presence or absence of undigested blood was determined.
Normal feces, %	Those feces that did not present any of the aforementioned alterations were considered normal.
LIAN1.1 Index	It is used as a single index of fecal quality and integrates the scoring of the different alternations observed, according to the following equation (Martinez and Vilchez, 2016): $$\text{LIAN1.1 Index} = \sum_{1}^{n} S_i \times \frac{25}{n}$$ Where "$\Sigma\ S_i$" is the summatory of the score (S) of each dropping sampled (i) in the same experimental unit, and "n" is the number of droppings sampled in the experimental unit. Score: 0, normal; 1, watery (according to the aforementioned method); 2, with undigested feed; 3, with desquamated mucosa; 4, bloody. Varies from 0 to 100, being higher with more abnormal feces observed.
Frequency of clinical pictures compatible with dysbacteriosis	The experimental unit was considered positive for dysbacteriosis if at least 30% of the feces were wet according to the aforementioned method (Panneman and Van Der Stroom-Kruyswijk, 2002).
Relative intestinal weight, %	At the end of the evaluation three chickens per experimental unit were sampled and the weight of the chicken and the intestine (without emptying the contents).
Intestinal content percentage, %	The sampled intestines were weighed after being emptied and washed. Values are expressed as the percentage of the total weight of the full intestine corresponding to intestinal contents.

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Data processing and statistical analysis: A Completely Random Design with four treatments and 10 replications was used. The experimental unit was the cage with seven birds. The presence of outliers was evaluated with Grubbs test (Grubbs, 1969) using the GraphPad Prism 5.03 software (Motulsky, 2007). Data were processed using SAS 9.2 software (SAS Institute, 2009). Variances of variables showing normal distribution were analyzed through GLM procedure of SAS software and treatment averages were compared through Duncan test (Duncan, 1955). Variances of variables without normal distribution (mortality and clinical evaluation of disbacteriosis) were analyzed with the non-parametric Kruskal-Wallis test (NPAR1WAY procedure with WILCOXON restriction) (Schlotzhauer and Littell, 1997; McDonald, 2009).

4. RESULTS

Results of the initial evaluation corroborate that the chicks in all treatments presented homogeneous conditions (Table 8), with no differences observed (P<0.05).

Table 8. Initial characteristics of chicks before providing treatments.

Variables [1]	Treatments [3]				P
	T1 (D) Standard diet	T2 (A) Low energy diet	T3 (B) Enzyme *on top*	T4 (C) Enzyme with matrix	
Maximum body weight (g)	51.0	51.4	49.9	49.9	0.6216
Minimum body weight (g)	41.3	41.8	41.5	42.3	0.1133
Range of weights (g)	9.74	9.55	8.31	7.62	0.1295
Average body weight (g) [2]	46.02	45.98	45.99	46.11	0.9368
Body weight standard deviation (g)	3.48	3.56	3.11	2.75	0.1933
Body weight variation coefficient (%)	7.56	7.71	6.76	5.98	0.1998

1 Presented values are average of 10 replications each.
2 Values of each replication are also average of seven individual values.
3 Codes used as part of the blind test protocol are indicated in brackets.
P Probabilities obtained from variance analysis. Is the probability (from 0 to 1) of error if declaring that at least one of the treatments is different from the others. The maximum value considered acceptable, by convention, is 0.05.

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In tables 9 to 11 the results of the study are presented. At the end of the evaluation if was found less weight and weight gain in treatment T2 than treatment T1 (P<0.02); however, no statistically significant differences among treatments T1, T3 and T4 (P>0.05) were observed in those variables. No differences were found in feed intake among treatments (P>0.05). Mortality was low y was not associated to treatments (P<0.28). Feed conversion ratio was higher for treatment T2 that T1 (P<0.01) and no statistically significant differences were observed among treatments T1, T3 and T4 (P<0.05). T2 showed a lower European efficiency index than T1 (P<0.01). Even though treatments T2 and T3 did not show statistically significant differences (P>0.05) in relation to T1 in the European efficiency index, T3 produced a higher value than T4 (P<0.05). No statistically significant differences were observed in the indicators used to evaluate the fermentation of intestinal content (P<0.56). The observed values of mortality and fecal quality are considered normal.

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Table 9. Indicators of growth observed in the evaluation.

Variables [1]	Treatments [3]				P
	T1 (D) Standard diet	T2 (A) Low energy diet	T3 (B) Enzyme *on top*	T4 (C) Enzyme with matrix	
Body weight at day 7, g/bird [2]	205.5	201.9	204.0	204.3	0.6423
Body weight at day 14, g/bird [2]	488.0 ab	473.5 b	492.0 a	485.7 ab	0.0887
Body weight at day 21, g/bird [2]	891.0 a	863.4 b	904.0 a	882.2 ab	0.0200
Body weight at day 28, g/bird [2]	1,475	1,448	1,485	1,464	0.4518
Body weight at day 35, g/bird [2]	2,048	1,998	2,068	2,061	0.1556
Body weight at day 42, g/bird [2]	2,649 a	2,565 b	2,689 a	2,629 ab	0.0166
Weight gain at day 7, g/bird	159.4	155.9	158.0	158.2	0.6025
Weight gain at day 14, g/bird	442.0 ab	427.5 b	446.0 a	439.6 ab	0.0769
Weight gain at day 21, g/bird	845.0 a	817.4 b	858.0 a	836.1 ab	0.0174
Weight gain at day 28, g/bird	1,429.0	1,401.9	1,439.0	1,418.1	0.4442
Weight gain at day 35, g/bird	2,001.6	1,952.4	2,021.8	2,014.8	0.1520
Weight gain at day 42, g/bird	2,603.0 a	2,519.4 b	2,643.0 a	2,583.1 ab	0.0159
Weight gain at day 7, g/bird/d	22.78	22.27	22.57	22.60	0.6025
Weight gain at day 14, g/bird/d	31.57 ab	30.54 b	31.86 a	31.40 ab	0.0769
Weight gain at day 21, g/bird/d	40.24 a	38.93 b	40.86 a	39.82 ab	0.0174
Weight gain at day 28, g/bird/d	51.03	50.07	51.39	50.65	0.4442
Weight gain at day 35, g/bird/d	57.19	55.78	57.77	57.57	0.1520
Weight gain at day 42, g/bird/d	61.98 a	59.99 b	62.93 a	61.50 ab	0.0159
Feed intake at day 7, g/bird	202.1	199.8	198.9	200.2	0.9591
Feed intake at day 14, g/bird	595.4	587.1	583.5	598.2	0.5219
Feed intake at day 21, g/bird	1,191.9	1,211.7	1,183.1	1,209.9	0.7169
Feed intake at day 28, g/bird	2,026.1	2,058.5	2,000.0	2,058.0	0.6779
Feed intake at day 35, g/bird	3,141.3	3,188.2	3,079.1	3,236.3	0.3381
Feed intake at day 42, g/bird	4,374.6	4,420.1	4,299.9	4,311.6	0.6530
Mortality week 1, %	0.00	0.00	0.00	0.00	1.0000
Mortality week 2, %	0.00	0.00	1.43	0.00	0.4040
Mortality week 3, %	1.43	0.00	0.00	0.00	0.4040
Mortality week 4, %	1.43	1.43	0.00	0.00	0.5780
Mortality week 5, %	0.00	0.00	1.43	0.00	0.4040
Mortality week 6, %	0.00	0.00	1.43	2.86	0.2829
Overall mortality, %	2.86	1.43	4.29	2.86	0.7629

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1 Presented values are average of 10 replications each.

2 Values of each replication are also average of seven individual values.

3 Codes used as part of the blind test protocol are indicated in brackets.

P Probabilities obtained from variance analysis. Is the probability (from 0 to 1) of error if declaring that at least one of the treatments is different from the others. The maximum value considered acceptable, by convention, is 0.05.

a,b Significance indexes obtained from Duncan test. Averages in a row sharing a same letter are not statistically different from each other (P>0.05). Rows with no letter present no statistically significant differences among the values in that row (P<0.05). The 0.05 value corresponds to the maximum acceptable probability of error, by convention, when declaring that differences between treatments do exist.

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Table 10. Indicators of efficiency observed in the evaluation.

Variables [1]	Treatments [2]				P
	T1 (D) Standard diet	T2 (A) Low energy diet	T3 (B) Enzyme *on top*	T4 (C) Enzyme with matrix	
Commercial feed conversion ratio at day 7	0.983	0.989	0.975	0.980	0.9397
Commercial feed conversion ratio at day 14	1.220	1.241	1.196	1.232	0.2492
Commercial feed conversion ratio at day 21	1.354 ab	1.403 a	1.316 b	1.371 a	0.0155
Commercial feed conversion ratio at day 28	1.401 ab	1.444 a	1.351 b	1.405 ab	0.0258
Commercial feed conversion ratio at day 35	1.558 ab	1.615 a	1.508 b	1.571 ab	0.0261
Commercial feed conversion ratio at day 42	1.671 b	1.737 a	1.642 b	1.684 ab	0.0077
Corrected feed conversion ratio at day 7	0.983	0.989	0.975	0.980	0.9397
Corrected feed conversion ratio at day 14	1.220 ab	1.241 a	1.186 b	1.232 a	0.0337
Corrected feed conversion ratio at day 21	1.338 bc	1.403 a	1.309 c	1.371 ab	0.0020
Corrected feed conversion ratio at day 28	1.374 ab	1.421 a	1.347 b	1.405 ab	0.0808
Corrected feed conversion ratio at day 35	1.534 ab	1.596 a	1.488 b	1.571 a	0.0272
Corrected feed conversion ratio at day 42	1.651 b	1.723 a	1.598 b	1.639 b	0.0011
European Efficiency Index at day 7	298.93	292.22	299.81	298.75	0.7392
European Efficiency Index at day 14	285.98 ab	273.16 b	292.55 a	281.99 ab	0.1061
European Efficiency Index at day 21	312.83 b	293.37 c	329.29 a	307.00 bc	0.0001
European Efficiency Index at day 28	378.34 ab	358.78 b	394.99 a	372.36 b	0.0054
European Efficiency Index at day 35	381.44 a	358.24 b	393.00 a	375.62 ab	0.0059
European Efficiency Index at day 42	382.18 ab	355.02 c	394.99 a	370.70 b	<0.0001

[1] Presented values are average of 10 replications each.

[2] Codes used as part of the blind test protocol are indicated in brackets.

P Probabilities obtained from variance analysis. Is the probability (from 0 to 1) of error if declaring that at least one of the treatments is different from the others. The maximum value considered acceptable, by convention, is 0.05.

a,b,c Significance indexes obtained from Duncan test. Averages in a row sharing a same letter are not statistically different from each other (P>0.05). Rows with no letter present no statistically significant differences among the values in that row (P<0.05). The 0.05 value corresponds to the maximum acceptable probability of error, by convention, when declaring that differences between treatments do exist.

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Table 11. Indicators of intestinal content fermentation.

Variables [1]	Treatments [3]				P
	T1 (D) Standard diet	T2 (A) Low energy diet	T3 (B) Enzyme *on top*	T4 (C) Enzyme with matrix	
Normal feces, %	79.20	82.60	81.00	80.40	0.8057
Watery feces, %	20.80	17.40	19.00	19.60	0.8057
Feces with undigested feed, %	1.58	1.60	1.53	1.75	0.9575
Feces with desquamated mucosa, %	0.58	0.55	0.65	0.50	0.8090
Bloody feces, %	0.00	0.00	0.00	0.00	-
LIAN1.1 Index	5.74	4.89	5.30	5.46	0.7948
Pictures compatible with dysbacteriosis, %	1 de 10	0 de 10	0 de 10	1 de 10	0.5616
Relative intestinal weight, % [2]	3.98	4.17	4.32	4.03	0.6948
Intestinal weight percentage, % [2]	24.76	26.34	26.37	24.37	0.6588

[1] Presented values are average of 10 replications each.

[2] Values of each replication are also average of three individual values.

[3] Codes used as part of the blind test protocol are indicated in brackets.

P Probabilities obtained from variance analysis. Is the probability (from 0 to 1) of error if declaring that at least one of the treatments is different from the others. The maximum value considered acceptable, by convention, is 0.05.

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5. CONCLUSIONS

At the end of the evaluation period, the obtained results allow to conclude the following:

1) The negative control diet with lower energy content (treatment T2) produced lower body weight, lower weight gain, higher feed conversion ratio and lower European efficiency index than standard diet (treatment T1) ($P<0.05$). No statistically significant differences were found in the other evaluated variables ($P<0.05$).
2) Feeding the evaluated product on top (treatment T3) produced no statistically significant effects ($P<0.05$) in the evaluated variables in comparison to standard diet (treatment T1).
3) The inclusion of the evaluated product applying an energy matrix (-100 kcal/kg of metabolizable energy in the feed; treatment T4) produced no statistically significant differences ($P>0.05$) in the evaluated variables in comparison to the standard diet (treatment T1).
4) Feeding the product on top (treatment T3) produced higher European efficiency index than applying an energy matrix (treatment T4). No statistically significant differences ($P>0.05$) were found in other evaluated variables.

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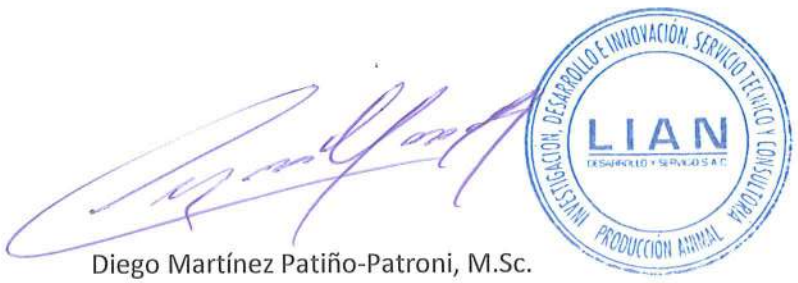
Lima, March 7th, 2017



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Evaluation of MANNANASE VTR in broilers:

Complementary data analysis

REPORT N° I10412017-5

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Evaluation of MANNANASE VTR in broilers: Complementary data analysis

REPORT N° I10412017-5

1. BACKGROUND

The evaluation of *MANNANASE VTR* in broilers was conducted and results were communicated in Report N° I10412017-3 on March 7th, 2017. The following is a complementary analysis of the data obtained, as Protecno Perú S.A.C. requested.

2. OBJECTIVE

The objective of the present analysis was to determine the probability of committing type I error, that is, to conclude that there are differences between treatments when in fact there are none, applied to the following aspects evaluated in the study:

- Effect of reducing the energy level in the diet.
- Effect of adding the evaluated product applying the energy matrix.
- Effect of adding the product on top (without applying an energy matrix).
- Differences between both adding strategies: on top and applying an energy matrix.

3. MATERIALS AND METHODS

The data obtained in the evaluation of MANNANASE VTR in broilers, communicated in Report N° I10412017-3, was used for the analysis. Treatments, as stated in that report, were the following:

- T1: Positive control (standard diet).
- T2: Negative control (same to T1 but with 100 kcal/kg less metabolizable energy).
- T3: Same to T1 but with 150 g/t *Mannanase VTR* added to the mix (on top feeding).
- T4: Same to T2 but with 150 g/t *Mannanase VTR* added to the mix (enzyme with matrix).

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The following variables were used in the analysis: body weight (g), weight gain (g/bird), feed intake (g/bird), commercial feed conversion ratio, corrected by mortality feed conversion ratio and European efficiency index. In order to determine the required probabilities, multiple average contrasts were carried out, independently in each case. In each case, a Completely Randomized Design with two treatments and 10 replications was used. The same average values per experimental unit obtained when processing data to elaborate report N° I10412017-3 were used. Data were analyzed using the GLM procedure of SAS 9.2. program.

4. RESULTS

In Table 1 are shown the error probabilities (P) in affirming that the observed difference (D) between each pair of treatments was produced by them and not by random factors. Thus, the probability of not making an error in such a declaration is 1-P. Both probability values can be expressed in decimal notation or in percentage. Thereby, for example, there is a probability of 91.82% [(1-P)x100] that the difference of 3.2% in corrected feed conversion ratio at day 42 between T3 (*on top*) and T1 (standard diet) is due to the treatments; and, as a consequence, an error probability of 8.18% (Px100) in such declaration, that is, the observed difference (3.2%) has been produced not by treatments but by any random factor (see Table 2).

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Table 1. Probabilities obtained for the different contrasts analyzed.

Variables	Comparisons							
	T2 versus T1 (Low energy versus standard diet)		T3 versus T1 (On top versus standard diet)		T4 versus T1 (Enzyme with matrix versus standard diet)		T3 versus T4 (*On top* versus enzyme plus matrix)	
	D [1]	P [2]	D [1]	P [2]	D [1]	P [2]	D [1]	P [2]
Body weight at day 7, g/bird [2]	-1.7%	0.1556	-0.7%	0.6193	-0.5%	0.6560	-0.2%	0.9224
Body weight at day 14, g/bird [2]	-3.0%	0.0570	0.8%	0.5735	-0.5%	0.7434	1.3%	0.4170
Body weight at day 21, g/bird [2]	-3.1%	0.0369	1.5%	0.2735	-1.0%	0.4832	2.5%	0.1049
Body weight at day 28, g/bird [2]	-1.8%	0.2649	0.7%	0.6942	-0.7%	0.6338	1.4%	0.3949
Body weight at day 35, g/bird [2]	-2.4%	0.1414	1.0%	0.5490	0.6%	0.6929	0.3%	0.8372
Body weight at day 42, g/bird [2]	-3.2%	0.0237	1.5%	0.3289	-0.7%	0.6035	2.3%	0.1509
Weight gain at day 7, g/bird	-2.2%	0.1241	-0.9%	0.6032	-0.8%	0.5947	-0.1%	0.9492
Weight gain at day 14, g/bird	-3.3%	0.0505	0.9%	0.5597	-0.5%	0.7247	1.5%	0.3972
Weight gain at day 21, g/bird	-3.3%	0.0340	1.5%	0.2637	-1.0%	0.4697	2.6%	0.0983
Weight gain at day 28, g/bird	-1.9%	0.2611	0.7%	0.6909	-0.8%	0.6271	1.5%	0.3887
Weight gain at day 35, g/bird	-2.5%	0.1389	1.0%	0.5459	0.7%	0.6926	0.3%	0.8335
Weight gain at day 42, g/bird	-3.2%	0.0229	1.5%	0.3261	-0.8%	0.5995	2.3%	0.1483
Feed intake at day 7, g/bird	-1.1%	0.7014	-1.6%	0.5929	-1.0%	0.7139	-0.6%	0.8399
Feed intake at day 14, g/bird	-1.4%	0.3685	-2.0%	0.3121	0.5%	0.7979	-2.5%	0.2720
Feed intake at day 21, g/bird	1.7%	0.4925	-0.7%	0.6901	1.5%	0.5585	-2.2%	0.3876
Feed intake at day 28, g/bird	1.6%	0.5671	-1.3%	0.6562	1.6%	0.5751	-2.8%	0.3147
Feed intake at day 35, g/bird	1.5%	0.3956	-2.0%	0.5589	3.0%	0.1507	-4.9%	0.1786
Feed intake at day 42, g/bird	1.0%	0.6388	-1.7%	0.5319	-1.4%	0.5477	-0.3%	0.9218
Commercial feed conversion ratio, d 7	0.6%	0.8030	-0.9%	0.7091	-0.4%	0.8631	-0.5%	0.8447
Commercial feed conversion ratio, d 14	1.7%	0.3187	-2.0%	0.3372	0.9%	0.5346	-2.9%	0.1771
Commercial feed conversion ratio, d 21	3.6%	0.0500	-2.8%	0.1355	1.2%	0.5351	-4.0%	0.0674
Commercial feed conversion ratio, d 28	3.0%	0.1539	-3.6%	0.1328	0.2%	0.8896	-3.8%	0.0826
Commercial feed conversion ratio, d 35	3.6%	0.1021	-3.2%	0.1422	0.8%	0.6641	-4.0%	0.0794
Commercial feed conversion ratio, d 42	4.0%	0.0429	-1.7%	0.3299	0.8%	0.6637	-2.5%	0.0609
Corrected feed conversion ratio, day 7	0.6%	0.8030	-0.9%	0.7091	-0.4%	0.8631	-0.5%	0.8447
Corrected feed conversion ratio, day 14	1.7%	0.3187	-2.8%	0.0535	0.9%	0.5346	-3.7%	0.0194
Corrected feed conversion ratio, day 21	4.9%	0.0067	-2.1%	0.1616	2.5%	0.1882	-4.5%	0.0263
Corrected feed conversion ratio, day 28	3.5%	0.1397	-1.9%	0.4665	2.2%	0.3003	-4.1%	0.0626
Corrected feed conversion ratio, day 35	4.0%	0.0044	-3.0%	0.2897	2.4%	0.1350	-5.3%	0.0959
Corrected feed conversion ratio, day 42	4.4%	0.0114	-3.2%	0.0818	-0.7%	0.6368	-2.5%	0.1992
European Efficiency Index at day 7	-2.2%	0.3161	0.3%	0.9089	-0.1%	0.9814	0.4%	0.9035
European Efficiency Index at day 14	-4.5%	0.1077	2.3%	0.4173	-1.4%	0.5396	3.7%	0.1991
European Efficiency Index at day 21	-6.2%	0.0098	5.3%	0.0502	-1.9%	0.4363	7.3%	0.0060
European Efficiency Index at day 28	-5.2%	0.0448	4.4%	0.1632	-1.6%	0.3936	6.1%	0.0349
European Efficiency Index at day 35	-6.1%	0.0100	3.0%	0.2206	-1.5%	0.5330	4.6%	0.1099
European Efficiency Index at day 42	-7.1%	0.0006	3.4%	0.0574	-3.0%	0.1027	6.6%	0.0059

[1] Percentage difference between both treatments.

[2] Probability obtained from the analysis of variances. Indicates the probability (from 0 to 1) of error when declaring that the treatments produce different effects.

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Table 2. Probabilities when making affirmations about the results obtained at day 42.

Affirmations	Probability of:	
	Not making error when affirming it [(1-P) x 100]	Making error when affirming it [P x 100]
That the reduction of energy in the diet (T2 versus T1) produced the following effects:		
"It reduced the body weight by 3.2%"	97.63%	2.37%
"It reduced the body weight gain by 3.2%"	97.71%	2.29%
"It increased the feed intake by 1.0%"	36.12%	63.88%
"It increased the commercial feed conversion ratio by 4.0%"	95.71%	4.29%
"It increased the corrected feed conversion ratio by 4.4%"	98.86%	1.14%
"It reduced the European efficiency ratio by 7.1%"	99.94%	0.06%
That feeding the product on top (T3 versus T1) produced the following effects:		
"It increased the body weight by 1.5%"	67.11%	32.89%
"It increased the body weight gain by 1.5%"	67.39%	32.61%
"It reduced the feed intake by 1.7%"	46.81%	53.19%
"It reduced the commercial feed conversion ratio by 1.7%"	67.01%	32.99%
"It reduced the corrected feed conversion ratio by 3.2%"	91.82%	8.18%
"It increased the European efficiency index by 3.4%"	94.26%	5.74%
That feeding the product with the energy matrix (T4 versus T1) produced the following effects:		
"It reduced the body weight by 0.7%"	39.65%	60.35%
"It reduced the body weight gain by 0.8%"	40.05%	59.95%
"It reduced the feed intake by 1.4%"	45.23%	54.77%
"It increased the commercial feed conversion ratio by 0.8%"	33.63%	66.37%
"It reduced the commercial feed conversion ratio by 0.7%"	36.32%	63.68%
"It reduced the European efficiency index by 3%"	89.73%	10.27%
That on top feeding versus with energy matrix (T3 versus T4) produced the following effects:		
"It increased the body weight by 2.3%"	84.91%	15.09%
"It increased the body weight gain by 2.3%"	85.17%	14.83%
"It reduced the feed intake by 0.3%"	7.82%	92.18%
"It reduced the commercial feed conversion ratio by 2.5%"	93.91%	6.09%
"It reduced the corrected feed conversion ratio by 2.5%"	80.08%	19.92%
"It increased the European efficiency index by 6.6%"	99.41%	0.59%

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