Iğdır Üniversitesi Fen Bilimleri Enstitüsü Dergisi
Iğdır University Journal of the Institute of Science and Technology

Testing Novel Eubiotic Additives: Its Health and Performance Effects in Commercially Raised Farm Animals

Sulhattin YAŞAR[1], İskender OKUTAN[2], Ramazan TOSUN[3]

ABSTRACT: A microbial fermentation process was developed to produce three eubiotic products containing probiotics, organic acids, enzymes, organic vitamins and minerals. The studies were carried out from 2015 to 2017. One product was specifically added with ground leaves of essential oil plant at the end of fermentation. As a result of this study, a dose of Eubiotic 1 ranging from 0.02 to 0.1% of diet remarkably increased egg yield and egg weight of laying hens. A daily increase of 1 to 3 kg milk per cow with no increase in dry matter intakes was obtained from a dose of 15 g eubiotic 1 per head per day in several farm trials. Calves had a 15 g Eubiotic 2 in warm milk grew faster than those given no supplement in milk. Calves and lambs having a severe level of diarrhea provided with 15 g supplement of eubiotic 2 in milk showed no sign of diarrhea after 6 or 24 h. Dairy cows and beef cattle having severe and acute metabolic disorders administrated with a dose of 75 g eubiotic 2 per cow per twice a day had no signs of such disorders in 24 h. Meat-type broiler chickens and quails given a diet of 0.5% supplement produced more profitable meat than those birds had no supplement. A 250 g live weigh gain with better feed conversion ratio (FCR) was obtained from the fattening beef cattle given a 20 g/day/head of eubiotic 2. Eubiotic mixture 3 was a silage additive and added to approximately 10.000 tonnes of corn silages and then the maturation of silage occurred in 15 to 30 days. It was concluded that these beneficial effects were mainly due to the combination of various active agents, and the proposed dosages were 0.5% in broiler diet, 200-1000 mg/kg of laying hens, 30 g/head in dairy cows.

Keywords: Animal nutrition, Eubiotic fed additives, microbial fermentation, health and performance

Yeni Eubiyotik Katkı Madde Testleri: Ticari Çiftlik Koşullarında Sağlık ve Verim Üzerine Etkiler

ÖZET: Bu çalışmada mikrobiyal fermentasyon ile canlı probiyotik, organik asit, enzimler ile organik kökenli vitamin ve mineral maddeler içeren üç yeni eubiyotik katkı maddesi geliştirilmiştir. Araştırmalar 2015-2017 yılları arasında yürütülmüştür. Bunlardan birisi, özellikle fermentasyon sonunda katılan esansiyel ekstrakt kaynağı olarak öğütülmüş bitki içermektedir. Yumurtacı tavukların karma yemlerine %0,02 ile %0,1 arasında eubiyotik 1 katkısının yumurta verimi ve yumurta ağırlığı önemli şekilde artırdığı görülmüştür. Farklı işletmelerde barındırılan süt sığırlarına aynı katkıdan günde 15 gram (bir dizi ön test sonuçlarına göre bu doz etkili olmuştur) yedirildiğinde ise 1-3 kg arasında inek başına süt verimi artışı sağlanmıştır. Yeni doğan buzağılara sütle 15 g yedirildiğinde daha hızlı büyüme sağlanmış, ishal olan kuzu ve buzağılarda ishal vakasına 6 ile 24 saat içerisinde rastlanılmamıştır. Metabolik rahatsızlıklara sahip ergin sığırlara günde iki kere 75 g'lık doz su veya süt ile verildiğinde hayvanlar eski sağlık durumlarına 24 saat içerisinde girmiştir. Eubiyotik 1 karşımından et tipi piliçlere %0.5-1.0 dozlarında rasyon ile verildiğinde ise yemden yararlanma ve büyüme hızı önemli derece artmıştır. Et sığırlarına günde 20 g eubiyotik 2 karışımı verildiğinde günlük canlı ağırlık artışında 250 g daha fazla kazanç sağlanmıştır. Eubiyotik 3 karışımı 10.000 ton mısır silajına katıldığında silajlar 15 ile 30 gün içerisinde olgunluğa ulaşmıştır. Tüm bu yararlı etkilerin bu yeni ürünlerin değişik aktif maddeler içermesinden kaynaklandığı sonucuna varılmıştır. Ayrıca, bu ürünler tamamen doğal kökenli ve hiçbir zararlı etkisine rastlanılmamış olup en faydalı dozların etlik piliçlerde rasyonun %0.5, yumurtacı rasyonlarında 200-1000 mg/kg, süt ineklerinde günde 30 g/baş, buzağı ve genç hayvanlarda 15 g/baş, metabolic rahatsızlıklarda 75 g/baş ve silaj katkı maddesi olarak ton başına 1 g olarak tespit edilmiştir.

Anahtar Kelimeler: Eubiyotik yem katkı maddeleri, hayvan besleme, mikrobiyal fermentasyon, sağlık ve verim

Cilt/*Volume:* 7, Sayı/*Issue:* 4, Sayfa/*pp:* 297-308, 2017
ISSN: 2146-0574, e-ISSN: 2536-4618 DOI: 10.21597/jist.2017.224

[1] Sulhattin YAŞAR (0000-0001-9334-1303), Iğdır Üniversitesi, Ziraat Fakültesi, Zootekni Bölümü, Iğdır, Türkiye
[2] İskender OKUTKAN (0000-0001-9334-1303), Gıda Tarım Hayvancilik Bakanlığı, Şarkıkaraağaç İlçe Müdürlüğü, Isparta, Türkiye
[3] Ramazan TOSUN (0000-0001-9334-1303), Sultanhanı Beldesi, Aksaray, Türkiye
Sorumlu yazar/Corresponding Author: Sulhattin YAŞAR, sulhattin.yasar@gmail.com

Geliş tarihi / *Received:* 02.10.2017
Kabul tarihi / *Accepted:* 27.10.2017

INTRODUCTION

Several numbers of feed additives have been tested as alternatives to feed antibiotics in animal nutrition to improve the health statues as well as to enhance the animal performance. However, there is no single alternative to the feed antibiotics or it is too difficult to develop a single alternative agent due to the fact that the animal health and performance, and subsequently their microbiota of gastrointestinal tract are under the great influence of the type of diets, environment, husbandry, managements and the strains of animal (Pan and Yu, 2014). The term "eubiotic nutrition" is an integrated strategy to combine different kinds of feed additives in order to achieve a healthy intestinal microbial flora by lowering pathogenic bacteria (dysbiosis) while increasing lactic acid bacteria (eubiosis) throughout the animals' digestive tract (Kim, 2017; Elala and Ragaa, 2015). This strategy can simply be in a form of a preparation of premixed active agents, whose individual efficacies in farm animals were proven (Kim, 2017). Generally, the synbiotics products with a mixture of pro- ad pre-biotics may provide eubiotic effects (Zhang et al., 2010; Pandey et al., 2015). Using organic acids with or without phytogenic additives as an eubiotic mixture (Ragaa and Korany, 2016; Polycarpo et al., 2017) has been successfully used an alternative to the feed antibiotics in meat-type poultry (Fascina et al., 2012). However, further formulations of synbiotic mixtures with organic acids, essential plant oils and feed grade enzymes may become too complex, and could not be fit for the purpose. These types of mixture may also contain pure chemical substances (organic acids, trace elements, essential oils) which could interfere with the remaining active agents (probiotics/ezymes). A pre-coating or capsulation of multiple active agents in a given additive preparation is crucial for the protection of active agents from the digestive secretions and for their mode of action in the specific sites of digestive tract. Elala and Ragaa (2015) clearly showed a eubiotic effect of organic acid by the supplementation of fish diets with potassium diformate, positively affecting gut health and animal performace.

To our best of knowledge there are no eubiotic mixtures produced by microbial fermentation containing several active agents in naturally protected form. Therefore, the aim of this research was to demonstrate the effects of three eubiotic mixtures on animal health and performance of several animal species under farm conditions.

MATERIALS AND METHODS

Method of Eubiotic Production

A solid state fermentation (SSF) technology was applied to produce three eubiotic mixtures from the use of agricultural waste products at the Suleyman Demirel University, in the department of Animal Science, Isparta Turkey. The SSF was carried out at 200 L of bioreactor, using GRAS (generally regarded as safe) bacteria and yeast, to ferment the biomass under fixed conditions (pH<0.5, intermitting stirring 10 min at every 12 h, moisture content >60%, temperature=35-39^{0}C), according to the modified method mentioned in the work (Yasar et al., 2016). A natural food material during the fermentation was added to coat the viable probiotic bacteria and yeast cells. All volatile and nonvolatile organic acids produced during the fermentation were also protected by food material. When an eubiotic mixture is intended to contain a source of essential plant oils a fine ground leaves of essential oil plant was added to the fermenting substrate at the end of fermentation, followed by a homogenous mixing process. The final wet product was immediately dried under 50 ^{0}C of temperature by the forced aerations for 48 hours, followed by a grinding (3 mm sieve) and mixing process (Yasar et al., 2016).

Formulation of Eubiotic mixtures

All three eubiotic mixtures contain averaged active live cells of *Saccharomyces cereviciae* around 2.1 x 10^9 cfu g^{-1} and *lactobacillus* spp. around 7.5 x 10^8 cfu g^{-1}. The stability of microorganisms in the product stored for 1 year under room temperature was an average of 92%. The recovery of yeast and bacteria microorganisms in simulated gastric acids was around 87%. All three products were subjected to a heat treatment at 60 ^{0}C for 15 min, 75 ^{0}C for 15 min and 110 ^{0}C for 15 min, consequently. The results showed that some species of *lactobacillus* spp. survived up to 110^{0}C, but no *Saccharomyces cereviciae* survived above 75 ^{0}C for 15 min. The product has a good smell and aroma of organic acids, mainly lactic acid (7%) and acetic acid (5% in dry matter). The final products contained an averaged activity of 2000 IU of xylanase, 800 IU of beta-glucanase and 450 IU of cellulase per gram dry matter. Stability of enzymes for 6, 12 and 24 months at room temperature were tested to be around 97, 96 and 95%,

respectively. The mixture with essential oil plants has only an amount of ground plant leaves around 0.5%. All eubiotic mixtures did not significantly differ in their formulations, the difference was only the ratio and type of agricultural waste product and food ingredients in total formulations. Eubiotic 1 has contained a source of essential plant oils and intended to be used in dairy cows and laying hens, whereas the eubiotics 2 and 3 did not contain any source of essential plant oils. Eubiotic 2 was intended to be used in meat-type poultry, calves and lambs. The eubiotic 3 was intended to be used as silage additive.

Animal trials with eubiotic mixture 1

Trials of Dairy cows: Four commercial dairy farms (Holstein-Friesen) in Turkey were selected from various locations (Atabey, Burdur, Şarkıkaraağaç) to conduct a feeding trial at different occasions. The cows were in mixed lactation periods. The feeding trial was applied to the cows in lactation periods, while the cows in late lactations were not included in the study. Two independent groups, one control and one test were prepared in the barns. The differences in age, days in lactation, the number of lactations and animal weight were insignificant ($p > 0.05$) between the control and test groups. Both groups of animals were fed on the same diets, mainly forage (ad libitum) and concentrate according to routine dietary management at the farms. The daily milk yield per cow was recorded for 7 days before the start of feeding trials for both groups. All the cows were fed twice a day after milking. The cows in the test groups were only given a dose of 15 g eubiotic mixture 1 per cow per meal by tapping to the concentrate just after the milking.

Trial of laying hens: This trial was conducted in Erzurum province at the poultry research unit of Ataturk University, Agricultural Faculty, Department of Animal Science, Erzurum Turkey. A total of 144, 65 week-old hens (Lohman LSL) with similar body weight were randomly divided into 36 replicate cages (50x46x46 cm), each with 4 hens. The hens were fed the same diet of the control (Table 1) and subjected to a lighting regime of 16 h per day prior to the trial. Six experimental diets were prepared in this study: each kg of a control diet formulated to meet the nutrient requirements of laying hens (NRC, 1994) was supplemented with 0, 200, 400, 600, 800 and 1000 mg of the eubiotic mixture 1. Each of 6 diets was randomly allocated to 6 replicate cages, each with 4 hens. All the hens had a free access to water and feed and subjected to a photoperiod of 16 h per day. A routine daily inspection of housekeeping was practiced throughout the trials. Feed intakes, egg weight, egg production, FCR (g feed per g egg produced) were recorded fortnights for each of replicate cages per treatment.

Animal Trials with Eubiotic Mixture 2

Clinical experiment with newborn calves and lambs: A number of veterinarians in the provinces of Isparta, Konya, Burdur and Aksaray were freely provided with the eubiotic mixture 2. These technical staffs have been long working on the region and had good reputations with their customers. During their routine farm visits, they have tested the effects of eubiotic mixture 2 to treat the newborn calves and lambs suffered from a severe diarrhea (microbial origin, not nutritional) and they compare their effects with the use of a commercial antibiotic which they routinely used for such treatment. This intervention study has been lasted for 2 years. The results of this experiment were presented as average number of days that the calves or lambs were recovered from a severe diarrhea compared to the days of recovery after an antibiotic treatment on the positive controls of calves in the same region during the same study period.

Beef cattle fattening trial: This trial was conducted in Isparta at a beef cattle farm. A total of 46 beef cattle at 12 month-age (Crossbreed of Simmental) was divided into 2 groups, one control and one test group. All the animals in both groups had a 10-day of adaptation period after receiving an anti-parasite medication before the start of fattening trial. All the animals were fed on a TMR (total mixed ratio) diet for 30 days. The cattle in test group were given a daily dose of 30 g eubiotic mixture 2, which was just tapped on the TMR. All the animals were weighed before and after the fattening period. Total feed intakes, weight gains and feed conversion ratio (FCR) were calculated for all animals. At the end of experiment, a blood sample was taken from all the animals and subjected to a blood chemistry analysis.

Broiler trial: Three isocaloric and isonitrgenous diets, C, T1 and T2 were added with 0, 5.0 or 10.0 g kg^{-1} of eubiotic mixture 1, respectively. The diets were formulated according to the NCR (1994) norms of nutrient requirements of broiler chicken. A total of 315 one-day-old Ross-PM 308 chicks were randomly distributed into 15 floor pens, each with 21 birds. The birds in 5 replicated flour pens were assigned to each of the dietary treatments and fed ad libitum for 42 days. The amount of consumed feed (feed intake, FI) of birds kept in each pen was daily recorded. The birds were weighted at the end of every week to measure individual body weight (BW). Weight gain (WG) and FCR were calculated from the weekly FI and BW values per pen.

Trial with Eubiotic mixture 3 as silage additive

Maize was harvested during the period of the middle dough maturity. The maize was chopped in 1.5 cm and ensiled in 1.5 L anaerobic jars. Two groups of silage materials were added with or without silage additive. Silage additive was dissolved in distilled water 0.1 g per 10 L and homogenously mixed before the application. The application rate was 1g of silage additive per 1000 kg fresh silage materials. The same amount water without silage additive was also sprayed into the control silage materials. Three jars from each group were sampled for chemical analysis 0,7, 15, 30 and 50 days after ensiling.

Statistical analysis: The data after subjected to a homogeneity test was analyzed by the analysis of variance (ANOVA) using a general linear model procedure (SPSS, 2006). The results were presented as means ± standard error. The significance of the differences between means was separated by Duncan's multiple range tests at $p < 0.05$.

RESULTS AND DISCUSSION

Experiments with Eubiotic Mixture 1

Providing the dairy cows in several locations in Turkey with a daily dose of 30 g Eubiotic Mixture 1(top-dressed) in several locations in Turkey significantly $p<0.05$ increased milk yield per cow per day in all 4 experiments, as compared to the cows given no supplement in the same farms (Table 1-4). Increased milk yield by the use of Eubiotic Mixture 1 did not associated with any increase in feed intake. The range of increased milk yield was from 0.5 to 4.0 kg per cow per day. During the withdrawing period of the Eubiotic Mixture 1 (Table 3) the milk yield was remained unchanged, as compared to the milk yield of control group.

Table 1. Effect of Eubotic Mixture 1 on milk yield of Holstein Friesian Cows at farm conditions

Experiment 1	**Control**	**Test**	
N	50	50	
Age, year	4.5-5.0	4.5-5.0	
Days in lactation	102	112	
Silage, kg DM/day	12.5	12.5	
Wheat straw, kg in DM day^{-1}	4	4	
Concentrate (19% CP) kg dry matter/day	9.0	9.0	
Eubiotic mixture 1, g day^{-1}	0.0	30	
Study period in days	7	7	
	Control	**Test**	**P**
(Average Milk yield in 7 days (kg per cow per day			
Before	23.8±0.75	23.4±0.20	0.342
During	$23.85±0.70^{a}$	$25.34±0.40^{b}$	0.010

[a-c] Different letters in the same column indicate significant ($P<0.05$) differences in the test parameter between the group means

The farmers did not tend to feed the dairy cows according to the milk yield, milk fat content and body condition scores at the regions.

Farmers were however very satisfied with the use of the supplement. In all the farms, the cows had watery droppings similar to the diarrhea during the first two days of the use of the Eubiotic Mixture 1. This result indicated that the Eubiotic Mixture 1 may enhance the utilization of nutrients in gastrointestinal system by improving the health of gut microflora due to the fact the eubiotic mixture 1 provided an appreciable levels of LAB (lactic acid producing bacteria), organic acids, essential plant oils and fiber degrading enzymes.

Table 2. Effect of Eubotic Mixture 1 on milk yield of Holstein Friesian Cows at farm conditions

Experiment 2	**Control**	**Test**	
N (number of cows)	45	45	
Age, year	5.5-6.0	5.5-6.0	
Days in lactation	135	130	
Sugar beet pulp, kg in DM day^{-1}	4.5	4.5	
Wheat straw, kg in DM day^{-1}	4	4	
Concentrate (19% CP) kg dry matter day^{-1}	9.0	9.0	
Eubiotic mixture 1, g day^{-1}	0.0	30	
Study period in days	10	10	
	Control	**Test**	**P**
(Average Milk yield in 10 days (kg per cow per day			
Before	23.6±0.49	24.0±0.31	0.162
During	$23.52±0.39^{a}$	$26.35±0.21^{b}$	0.001

[a-c] Different letters in the same column indicate significant (P<0.01) differences in the test parameter between the group means

Several other eubiotic supplements, mainly feed enzymes improved feed digestibility of corn based diets in dairy cows and increased the daily milk yield (Gencoglu et al., 2010; Weiss et al., 2011). Supplementation of dairy ration with yeast products may have beneficial effects on hot seasons to increase dry matter (DM) intake and milk production (Moallem et al., 2010).

In contrary, in UK, the results of a field study showed that there were no statistical effects of yeast supplements on the performance of dairy cows under commercial conditions (Ambriz-Vilchis et al., 2017). But, it was recommended that the beneficial effects may be seen with the farms where a high level of concentrate feed is used, similar to the cases in our experiments. On the other hands, all the farms in our study are almost identically similar in terms of feeding regimes, climate, animal breeds and type of concentrates and forages, thus the beneficial effects were easily seen.

Table 3. Effect of Eubotic Mixture 1 on milk yield of Holstein Friesian Cows at farm conditions

Experiment 3	**Control**	**Test**	
N (number of cows)	25	25	
Age, year	5.5-6.0	5.5-6.0	
Days in lactation	150	175	
Sugar beet pulp, kg DM day^{-1}	5	5	
Wheat straw, kg DM day^{-1}	4	4	
Concentrate (19% CP) kg dry matter/ day	10	10	
Eubiotic mixture 1, g day^{-1}	0.0	30	
Study period in days	10	10	
	Control	**Test**	**P**
(Average Milk yield in 10 days (kg per cow per day			
Before	20.1±0.23	20.5±0.15	0.125
During	20.25±0.20^{a}	24.45±0.60^{b}	0.001
After	20.0±0.23	21.8±0.70	0.178

$^{a-c}$ Different letters in the same column indicate significant (P<0.001) differences in the test parameter between the group means

Table 4. Effect of Eubotic Mixture 1 on milk yield of Holstein Friesian Cows at farm conditions

Experiment 4	**Control**	**Test**	
N (number of cows)	40	40	
Age, year	4.5-5.0	4.5-5.0	
Days in lactation	105	110	
Wheat straw, kg dry matter/day	2.0	2.0	
Corn silage, kg dry matter/day	8.0	8.0	
Concentrate (19% CP) kg dry matter/day	9.0	9.0	
Eubiotic mixture 1, g day^{-1}	0.0	30.0	
Study period in day	90	90	
	Control	**Test**	**P**
days- Average Milk Yield kg per cow per day 90			
Before	18.0±0.90	19.2±0.55	0.114
During	20.20±0.41^{a}	25.75±0.57^{b}	0.0001

$^{a-c}$ Different letters in the same column indicate significant (P<0.001) differences in the test parameter between the group means

Overall, it can be concluded that this novel Eubiotic Mixture 1 has beneficial effects in increasing milk yield and improving cows' overall health because it contained several types of active agents having beneficial effects on gut flora restoration, enhanced nutrient digestion and assimilation. Supplementation of broiler diets with eubiotic mixture 1 significantly ($p<0.05$) improved weight gains and feed conversion ratio (FCR) in 21 and 42 days old broiler chickens (Table 5).

Table 5. Effect of Eubotic Mixture 1 on the performance of Ross PM 308 broiler chicks at farm conditions

Treatments	Control	Test 1 (0.5%)	Test 2 (1.0%)	SEM (Standart Error of Mean)
n	63	63	63	---
BW, g bird^{-1}				
d 0	54.7	54.8	55.0	0.50
d 21	907^{b}	968^{a}	964^{a}	19.0
d 42	2276^{b}	2406^{ab}	2502^{a}	72.1
WG, g bird^{-1}				
d 21	852^{b}	914^{a}	909^{a}	18.9
d 42	2221^{b}	2351^{ab}	2447^{a}	71.8
FI, g bird^{-1}				
d 21	1561	1569	1557	33.0
d 42	4890	4934	5088	104.0
FCR				
d 21	1.83^{b}	1.72^{a}	1.72^{a}	0.04
d 42	2.20^{b}	2.10^{ac}	2.08^{a}	0.03
Deads (0-42 days)	1	3	3	---

$^{a-c}$ Different letters in the same column indicate significant ($P<0.05$) differences in the test parameter between the group means

Promoted growth rate and improved FCR are in good agreement with the results obtained from the probiotics products of yeast origin (Haldar et al., 2011; Reisinger et al., 2012; Yasar and Desen, 2014). In contrary there was no influence of 0.5 and 1.0% supplementation of a probiotic additive (protexin) neither on growth performance nor carcass yield in the broilers of Japanese quails (Ayasan, 2016). However, the same additive significantly increased only the body weight of broiler chicken (Fallah, 2016). This improvement did not associated with any increase in feed intake (FI) of the broiler chickens in this experiment. In generally, the monogastric animals did not increase their FI with the diets supplemented with probiotic, prebiotics and essential plant oils (Fallah, 2016; Gao et al., 2008; Sarica et al., 2009). Supplementing the diets with 0.5 and 1.0% of eubiotic mixture 1 at commercial condition is highly recommended to enhance the growth rate.

The hens fed on the diets supplemented with 0 mg kg^{-1} to 1000 mg/kg eubiotic mixture 1 consumed almost similar amount of feed throughout the experimental period since there were no significant ($P>0.05$) differences in FI between all the groups (Table 6). The overall FI ranged from 149.02 to 150.41 g (SEM = 0.24) per hen per day in this trial. The effect of dietary

supplementation on the egg production was significant (P<0.05). There were significantly (P<0.05) high egg production values obtained from the 800 and 1000 mg kg^{-1} groups; 93.75 and 96.66%, respectively, as compared to the value of 89.23% obtained from the 0 mg kg^{-1} group. However, the increases in the egg production values (90.41 to 92.70%, with a SEM of 0.51) in the 200, 400 and 600 mg kg^{-1} eubiotic feed additive (EFA) groups did not significantly (P>0.05) differ from the value (89.23%) in the 0 mg kg^{-1} EFA group. The egg weight of 64.13 g/hen obtained from the control group (0 mg kg^{-1} EFA) was significantly (P<0.05) increased to the egg weights of 69.25 and 67.34 g hen^{-1} obtained from the 200 and 1000 mg kg^{-1} EFA groups. Increases in egg weight obtained from the remaining EFA supplemented groups did not significantly (P>0.05) differ from the egg weight of the control group. Furthermore, a 2.62 value of FCR obtained from the control group was significantly (P<0.05) different from the 2.32 to 2.49 values of FCR obtained from the 200 to 1000 mg/kg EFA groups, whose FCR values did not differ significantly (P>0.05) from each other. As overall, there was significant improvement (P<0.05) in FCR of the hens fed on the diets with 200 to 1000 mg kg^{-1} eubiotic mixture 1 in this study.

Table 6. Effects of varying supplementation levels of Eubotic Mixture 1 on laying performance of Lohman LSL hens

Inclusion, mg kg^{-1}	Feed intake (g hen^{-1})	Egg weight (g hen^{-1})	Egg production (% of kept hens)	FCR (g:g)
0	149.02	64.13^{c}	89.23^{c}	2.62^{a}
200	148.77	69.25^{a}	90.41^{bc}	2.39^{bc}
400	148.11	65.58^{bc}	92.70^{bc}	2.45^{bc}
600	148.15	64.77^{c}	92.08^{bc}	2.49^{ab}
800	148.54	64.70^{c}	93.75^{ab}	2.46^{bc}
1000	150.41	67.34^{ab}	96.66^{a}	2.32^{c}
SEM*	0.24	0.31	0.51	0.02
Probability (P)	>0.05	=0.000	=0.000	=0.000

$^{a-c}$ Different letters in the same column indicate significant (P<0.05 or P<0.001) differences in the test parameter between the group means. *SEM; Standard error of means

In this study, voluntary FIs of the hens were not influenced by dietary eubiotic mixture 2 supplementation. This result obtained from our study confirmed the previous results: under normal practical conditions, the beneficial effects of dietary supplementation of microbial feed additives (probiotics/eubiotics) in several poultry species were not regulated by any changes in voluntary FI (Gao et al., 2008; Sarica et al., 2009), but they have a significant effect on the alleviation of reduced FI and depressed birds' performance under subclinical infections (Kulshreshtha et al., 2017; Jiang et al., 2010). Therefore, the enhanced laying performance by the eubiotic mixture 1 supplementation in our trial was not obtained by the regulation of FI of the hens, but it could rather be due to their probiotic/eubiotic effects at the sites of digestion and absorptions improving digestibility and uptake of nutrients.

Experiment with Eubiotic Mixture 2

Eubiotic mixture 2 was specifically formulated to balance the microbial flora of gastrointestinal tract in the calves and to promote the growth rate in young growing cattle. The experimental design of fattening trial was shown in Table 7. The results showed that the cattle in the test group receiving a daily dose of 30 g eubiotic mixture 2 per head significantly grew and utilized the feed better than

the cattle in the control group receiving no additives (Table 8). Less amount of dry matter intake in the test group cattle indicated that the eubiotic mixture 2 caused a better nutrient assimilation at the sites of digestion and absorption, compared to the cattle receiving none supplements. The mode of action of this supplement needs to be further investigated, while the most of important action would supposedly be the enhanced lactic acid bacteria population and their consecutive benefits in the digestive systems. In the rumen the changes in microbial flora are also needed to be investigated.

There have been a number studies showing the growth promoting effects of probiotics in young beef cattle, similar to the our case (Seymour et al., 1995, Beharka et al., 1990; Ely et al., 1990; Hughes, 1988; Fallon and Harte, 1987). In these studies, it was shown that the probiotics may maintain a good balance of gut microbiota, leading better absorption and assimilation of nutrients in fast growing animals.

Table 7. Breed, age, initial weights, dry matter (DM) requirements and fattening period (days)

	Control	**Test**
Age, month	12.0±2.0	11±3.0
Breed	Simmental Cross Breed	Simmental Cross Breed
N (number of cattle)	17	29
Initial weight, kg	375.65±54.0	387.97±63.2
Period, day	30	30
DM intake, kg per day	11.64	12.24
Concentrate, kg DM per day	6.03 (15%CP – 2600 kcal/kg ME)	5.94 (14% CP –2700 kcal/kg ME)
Forage, kg DM per day	4.77	4.095
Molasses, kg DM per day	0.39	0.39
Vitamin+Mineral, kg DM per day	0.09	0.09
Eubiotic 2, g per head per day	0.000	30

Table 8. Effect of Eubiotic mixture 2 on the fattening performance of Simmental Cross breeds

	Control	Test
N	17	29
Initial weight, kg	375.65±54.0	387.97±63.20
Day in fattening	30	30
Final weight, kg	403.53±55.85	428.69±68.43
Averaged weight in fattening, kg	388.00±55.00	408±65.75
DM, Requirement, kg day^{-1}	11.64	12.24
DM intake, kg day^{-1}	11.28	10.555
Average daily gain, kg	1.062±0.140a	1.360±0.120b
Average feed conversion ratio	10.61	7.76

[a-c] Different letters in the same column indicate significant (P<0.05) differences in the test parameter between the group means

In this study, the results of blood chemistry (EC 98/58) showed that the cattle fed on the diet with eubiotic mixture 2 had significantly lower levels of blood creatinine and urea than the cattle fed on the diet with no additive. In table 10, the results of field study (several locations in Turkey) indicated that the eubiotic mixture 2 is very effective to prevent the diarrhea as early as possible, compared to any commercial antibiotics used for the treatment. These calves also grew faster than the control calves receiving no supplement at all (Table11).

Table 9. Blood chemistry of beef cattle fed on the control and test groups

	CREA	**UREA**	**TBIL**	**DBIL**	**TP**	**ALB**
Control	1.99±0.39	70**±9.**68	0.37**±0.**10	0.49**±0.**60	8.80±0.30	3.63±0.22
Test	1.52±0.09	57.47**±0.**72	0.35±0.13	0.51±0.12	8.33**±0.**57	3.34**±0.**06
P	0.049	0.034	**P>0.05**	**P>0.05**	**P>0.05**	0.042

[a-c] Different letters in the same column indicate significant (P<0.05) differences in the test parameter between the group means

Table 10. Prevention of severe diarrhea in calves and lambs by a daily treatment of Eubiotic Mixture 2

	Eubiotic mixture 2 treatment	**Antibiotic treatment**
Number of farms	210	150
Number of calves/lambs	750/120	536/105
Averaged age of calves/lambs (days)	7.1±2.0/5.0±0.7	6.5±2.7/4.8±0.6
Days in diarrhea	3.1±0.5/2.0±0.3	4.0±1.2/3.0±0.5
Days of recovery	1.5±0.70a/0.5±0.5a	5.5±2.3b/2.5±0.3b
Visual appraisal (General health, skin and hair, vitality)	++++/+++	++/+

[a-c] Different letters in the same column indicate significant (P<0.05) differences in the test parameter between the group means

All these results clearly showed that the beef cattle, young calves and newborn calves could be healthier and grow faster with the diet containing eubiotic mixture 2 at commercial farm conditions. It seemed that the use of eubiotic mixture 2 containing several types of active agents (probiotics, enzymes, organic acids etc.,) may induce remarkable growth promoting effects. This result confirmed all the results obtained from previous studies regarding the use of individually supplemented probiotics, enzymes and organic acids in similar cases (Ambriz-Vilchis et al., 2017; Yasar et al., 2016; Ayaşan, 2016; Elala and Ragaa, 2015; Yasar and Desen 2014; Fascina et al., 2012).

Table 11. Effects of Eubiotic Mixture 2 on the growth performance of calves

	Control	**Test**	**P**
N (number of calves)	22	22	
Initial weights, kg	84.5±1.55	81.2±2.10	0.234
Days in feeding test	7	7	
Average daily weight gain per calve	0.585±0.21a	0.935±0.22b	0.045
Visual appraisal (General health, skin and hair, vitality)	++	+++	

[a-c] Different letters in the same column indicate significant (P<0.05) differences in the test parameter between the group means

Silage additive

Table 12. Chemical composition of maize silage treated with or without an inoculant of Eubiotic Mixture 3 (percentage of DM, Dry matter)

Days in ensiling	Treatments	DM loss%	pH	Lactic acid	Acetic acid	Butyric acid
0	Control	0	6.4± 0.1	0.65± 0.01	0.31± 0.11	0.0
	Test	0	6.2± 0.1	0.68± 0.07	0.33± 0.07	0.0
7	Control	0.56± 0.01a	5.5± 0.1a	0.90± 0.08a	0.75± 0.005a	0.0
	Test	0.38± 0.15b	5.1± 0.1b	1.56± 0.15b	0.55± 0.07b	0.0
15	Control	2.56± 0.21a	5.6± 0.1a	1.23± 0.17a	1.73± 0.32a	0.0
	Test	0.52± 0.05b	4.4± 0.1b	4.55± 0.95b	0.64± 0.04b	0.0
30	Control	3.1± 0.33a	5.0± 0.1a	2.50± 0.73a	1.68± 0.25a	0.0
	Test	0.70± 0.5b	4.0± 0.1b	5.18± 1.15b	1.00± 0.06b	0.0
50	Control	3.10± 0.75a	4.8± 0.1a	3.81± 0.05a	1.22± 0.005a	0.0
	Test	0.66± 0.25b	3.9± 0.1b	5.55± 0.08b	0.96± 0.04	0.0

[a-c] Different letters in the same column indicate significant (P<0.05) differences in the test parameter between the group means

Eubiotic mixture 3 was used as silage additive. In our in vitro ensiling experiment, the maize silage added with an inoculant had significantly shorter maturation during the ensiling process, compared to the maize silage (Table 12). The loss of dry matter was significantly lower with the use of silage additive, similarly the lower pH values were observed. The levels of lactic acids in the inoculated silage samples were significantly higher. Our on-going research on the field with 10 000 tonnes of maize and alfalfa silage prepared with addition of an 1 g eubiotic3 per ton in Isparta and Erzurum province clearly indicated that the customers were very satisfied with the use of eubiotic mixture 3 as silage additive due to the fact that the silage was of good quality and its aerobic stability in commercial conditions is longer than they have ever observed. Our laboratory analysis is stil on-going to investigate the nutritional and quality changes in these silage samples.

CONCLUSION

We have clearly observed important beneficial effects of these novel eubiotic mixtures for better animal performance. These effects were mainly due to the combination of various active agents (organic acid, probiotics, enzymes, antioxidants, vitamins and in some cases essential oil plants).

Acknowledgement

Authors would like to thanks Dr. Barış EREN from the Department of Crop Science at Igdir University, for his help for the editorial help of the manuscript.

REFERENCES

Ambriz-Vilchis V, Jessop NS, Fawcett, RH, Webstre M, Shaw DC, Walker N, Macrea Al, 2017. Effect of yeast supplementation on performance, rumination time, and rumen pH of dairy cows in commercial farm environments. J Dairy Sci., 7:5449-5461

Ayaşan T, 2016. Efficacy of probiotic supplementation on growth performance and carcass traits in Japanese Quails (Coturnix coturnix Japonica). Indian J Anim Sci, 86: 795-798.

Beharka AA, Nagaraja TG, Morrill JL, 1990. Ruminal microbial and metabolic development in young calves fed calf starter supplemented with *Aspergillus oryzae*extract. J. Dairy Sci., **73** (Suppl. 1), 220.

Elala N. M. A. and Ragaa N. M., 2015., Eubiotic effect of a dietary acidifier (potassium diformate) on the health status of cultured Oreochromis niloticus, In Journal of Advanced Research, 6: 621-629.

Ely DG, Ragland KK, Johns JT, Aaron DK, 1990. Amaferm supplementation to steers grazing tall fescue. J. Anim. Sci., 68 (Suppl. 1), 31.

Fallah R, 2016. Productive performance, carcass trait and blood parameters of broiler chickens fed different levels of dried whey and protexin probiotic. International Journal of Basic Sciences & Applied Research, 5: 240-247

Fallon RJ, Harte FJ, 1987. The effect of yeast culture inclusion in the concentrate diet on calf performance. J. Dairy Sci., **7**0 (Suppl. 1), 119.

Fascina, Vitor Barbosa, Sartori, José Roberto, Gonzales, Elisabeth, Carvalho, Fabyola Barros de, Souza, Ivan Mailinch Gonçalves Pereira de, Polycarpo, Gustavo do Valle, Stradiotti, Ana Cristina, & Pelícia, Vanessa Cristina., 2012. Phytogenic additives and organic acids in broiler chicken diets. Revista Brasileira de Zootecnia, 41: 2189-2197

Gao J, Zhang HJ, Yu SH, Wu SG, Yoon I, Quigley J, Gao YP, Qi GH, 2008. Effects of yeast culture in broiler diets on performance and immunomodulatory functions. Poultry Science 87:1377-1384.

Gencoglu HRD, Shaver W, Steinberg J, Ensink LF, Ferraretto S. J, Bertics JC, Lopes MS, 2010. Akins Effect of feeding a reduced-starch diet with or without amylase addition on lactation performance in dairy cows. J. Dairy Sci. 93:723–732

Haldar S, Ghosh TK, Toshiwati, Bedford MR, 2011. Effects of yeast (Saccharomyces cerevisiae) and yeast protein concentrate on production performance of broiler chickens exposed to heat stress and challenged with Salmonella enteritidis. Animal Feed Science and Technology, 168:61-71.

Hughes J, 1988. The effect of a high strength yeast culture in the diet of early weaned calves. Anim. Prod., 46: 526.

Jiang Z, Mohln M, Schatzmayr G, Applegate TJ, 2010. Net effect of an acute phase response – partial alleviation with probiotic supplementation. Poult. Sci. 89:28-33.

Kim, I. H., 2017. (23/10/2017 accessed date). https://www.dsm.com/content/dam/dsm/anh/en_US/documents/Combinations_of_different_Eubiotics_do_they_work.pdf

Kulshreshtha G, Rathgeber B, MacIsaac J, Boulianne M, Brigitte L, Stratton G, Thomas NA, Critchley AT, Hafting J and Prithiviraj B., 2017 Feed Supplementation with Red Seaweeds, Chondrus crispus and Sarcodiotheca gaudichaudii, Reduce Salmonella Enteritidis in Laying Hens. Front. Microbiol (open journal). 8:567. doi: 10.3389/fmicb.2017.00567

Lee KW, Everts H, Beynen AC, 2004. Essential Oils in Broiler Nutrition. International Journal of Poultry Science 3: 738-752.

Moallem U, Lehrer H, Livshitz L, Zachut M, Yaboly S, 2010. The effects of live yeast supplementation to dairy cows during the hot season on production, feed efficiency, and digestibility. Journal of Dairy Science, 92: 343-351.

NRC, 1994. Nutrient Requirements of Poultry. 9th rev. ed. National Academic Press, Washington, DC, USA.

Pan D, Yu Z, 2014. Intestinal microbiome of poultry and its interaction with host and diet. Gut Microbes, 5: 108–119. http://doi.org/10.4161/gmic.26945.

Pandey KR, Naik S R, Vakil BV, 2015. Probiotics, prebiotics and synbiotics- a review. Journal of Food Science and Technology, 52: 7577–7587.

Polycarpo G.V, Anretta I, Kipper M, Cruz-Polycarpo V C, Dadalt J C, Rodrigues P H M, Albuquerque R, 2017. Meta-analytic study of organic acids as an alternative performance-enhancing feed additive to antibiotics for broiler chickens. Poult Sci., 96: 3645-3653.

Ragaa and Korany, 2016. Studying the effect of formic acid and potassium diformate on performance, immunity and gut health of broiler chickens. Animal Nutrition, 2: 296-302

Reisinger N, Ganner A, Masching S, Schatzmayr G, Applegate TJ, 2012. Efficacy of a yeast derivative on broiler performance, intestinal morphology and blood profile. Livestock Science, 143:195-200.

Sarica S, Corduk M, Yarim GF, Yenisehirli G, Karatas U, 2009. Effects of novel feed additives in wheat based diets on performance, carcass and intestinal tract characteristics of quail. South African Journal of Animal Science, 39:144-157.

Seymour WM, Nocek, JE. Siciliano-Jones J, 1995. Effects of a colostrum substitute and of dietary brewer's yeast on health and performance of dairy calves. Journal of Dairy Science, **78**: 412–420.

SPSS, 2006. Computer software SPSS Inc. Headquarters, Wacker Drive, Chicago, Illinois, USA.

Weiss WP, Steinberg W, Engstrom MA, 2011. Milk production and nutrient digestibility by dairy cows when fed exogenous amylase with coarsely ground dry corn. Journal of Dairy Science, 94: 2492-2499

Yasar S, Desen E, 2014. Efficacy of a feed probiotic bacteria (Enterococcus faecium NCIMB 10415), spore (Bacillus subtilis ATCC PTA-6737) and yeast (Saccharomyces cerevisiae) in Japanese quails. Bulletin UASVM Animal Science and Biotechnology, 71:63-70.

Yasar S, Gok M S, GurbuzY., (2016). Performance of broilers fed raw or fermented and redried wheat, barley, and oat grains. Turk J Vet Anim Sci., 40: 313-322.

Zhang MM, Cheng JQ, Lu YR, Yi ZH, Yang P, Wu XT. 2010. Use of pre-, pro-and synbiotics in patients with acute pancreatitis: a meta-analysis. World J Gastroenterol: WJG. 16: 3970.