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# Effect of postbiotic based on lactic acid bacteria on semen quality and health of male rabbits

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**Simple Summary:** Postbiotics, especially those derived from metabolites of *Lactobacillus*, have being proposed as an alternative to the use of antibiotics for prevention and treatment of some diseases. This study was performed in rabbits due to its economic importance as livestock species in the Mediterranean countries, as well as being experimental model in biomedicine. In this work, the use of a diet enriched with a postbiotic based on lactic acid bacteria is proposed to improve the seminal characteristics of the rabbit and its health.

**Abstract:** The aim of this study was to evaluate the effect of postbiotic based on lactic acid bacteria supplementation on semen characteristics and hematological and biochemical profile in rabbits. A total of 28 males were randomly allocated into two groups. Males received a Control diet and Enriched diet supplemented with postbiotic during 15 weeks. Body weight, feed intake and semen characteristics was recorded weekly. Hematological profile was recorded at the beginning and at the end of the experiment and biochemical profile at 0, 5, 10 and 15 weeks. Bayesian methodology was used for the statistical analysis. Feed intake was higher in Control diet (125.2 g) than in the Enriched diet (118.6 g, P = 1.00). The percentage of abnormal spermatozoa were higher in Control diet than in Enriched diet (30 % and 22 %; P = 0.93) and the percentage acrosome integrity was lower (97 % and 96 %; P = 0.87). The hematological profile was within the range of healthy rabbits. The plasmatic level of alanine aminotransferase was higher in Control diet than Enriched diet at 5 and 10 week (P = 0.93 and P= 0.94, respectively) and alkaline phosphatase was similar in Control diet along the experiment but it decreased in Enriched diet (P = 0.97). No difference was found in kidney parameters (uric nitrogen and creatinine). Enriched diet showed a higher total protein and globulin than Control diet (P = 0.99). Phosphorus was lower (P = 0.92) in Control diet than in Enriched diet. In conclusion, the addition of the postbiotic based on lactic acid bacteria seems to improve the quality of the semen and the liver profile in rabbits.

**Keywords:** Fermented food; Hepatic profile; Lactic acid bacteria; Postbiotic, Rabbit; Semen profile

## 1. Introduction

Probiotics are live microorganisms that when administered in adequate amounts confer health benefits on the host [1]. Probiotic microorganisms are primarily lactic acid-producing bacteria of the genus *Lactobacillus* [2]. These probiotics can regulate the balance of gut microbes, promote the growth and productivity of animals, and improve the host resistance to diseases [3]. Thus, they have been extensively used in dairy cattle [4], beef cattle [5], pigs [6], hens [7] and rabbits [8]. Postbiotics are defined as soluble products or metabolites secreted by probiotics that have physiological benefits to the host [9]. Postbiotics consist of a wide range of effector molecules [10] and they are capable of reducing the gut pH and, in turn, inhibiting the proliferation of opportunistic pathogens in the feed and gut microbiota [10, 11]. Postbiotics, especially those derived from metabolites of *Lactobacillus*, have being proposed as an alternative to the use of antibiotics not only in human but also in monogastric [12]. Currently the application of postbiotics in human food, animal fed and pharmaceutical industries is increasing and postbiotics products derived from *Lactobacillus* species are commercially available for prevention or treatment of some diseases [10].

Rabbit is a livestock species reared either for the production of meat, hair or skin or as an experimental reference for other species, such as pigs or humans [13]. In rabbit meat production, artificial insemination is being widely used in intensive production farms [14]. The success of rabbit's artificial insemination program depends to both a great extent on male health and reproductive performance [15]. Thus productivity, welfare and health of males should be improved by handling or feeding. Unlike other monogastric animals, data regarding the use of the postbiotics in rabbits are quite scarce [12]. The objective is to study the effect of postbiotic based on lactic acid bacteria supplementation on semen characteristics and hematological and biochemical profile in male rabbits.

## 2. Materials and Methods

### 2.1. Ethics statement

All experimental procedures were approved by the Miguel Hernández University of Elche Research Ethics Committee, according to Council Directives 98/58/EC and 2010/63/EU (reference number 2019/VSC/PEA/0163).

### 2.2. Product description

The fermented food product tested was the result of a specific process of fermentation of a substrate and a combination of specific lactic acid bacteria and yeast. Substrate was a plant-based food product primarily composed by soya, alfalfa and wheat with other minor components. The fermented food product contained the phylum Firmicutes (38.7 %), Proteobacteria (26.7 %), Bacteroidetes (18.3 %), Actinobacteria

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(14.5%) and TM7 (1.8%). At genus level *Lactobacillus* was the predominant, accounting for more than 6% of identified species [16].

### 2.3 Animals

A total of 28 rabbit males were used [17]. Males were held on the experimental farm at the Universidad Miguel Hernández de Elche (Spain). All animals were reared in individual cages (37.5 × 33 × 90 cm) during all the experiment. The photoperiod was 16 hours light: 8 hours dark.

### 2.4. Diets

Two diets were used. The Control diet presented the following composition: 17% crude protein, 15% crude fiber, 9% crude ash, 3.6% crude fat, 1.2% calcium, 0.6% phosphorus and 0.3% sodium. The Enriched diet presented the same composition supplemented with 2.0 kg of the fermented food product in a ton of feed.

### 2.5. Experimental design

Males were randomly divided into 2 groups of 14 males each, one group received the Control diet and the other the Enriched diet. Males had an adaptation period to the feed of 4 weeks. Experiment procedure lasted 11 weeks. Male body weight and feed intake were recorded weekly (Fig. 1).

### 2.6. Semen collection and evaluation

Two ejaculates per male were collected each week on a single day an artificial vagina, with a minimum of 30 min between ejaculate collections. After adaption period, semen evaluations were performed during 11 weeks. If gel was present, it was removed. Only ejaculates exhibiting a white color were classified as normal and were evaluated. Ejaculates were diluted (dilution 1:5) with TRIS–citrate-glucose extender. Percentages of motile sperm evaluated subjectively (from 0 to 5) under a microscope at a magnification of 400x with thermostated plate set at 37ºC.

An aliquot from each ejaculated (0.1 ml) was fixed with 0.9 ml of 2% glutaraldehyde solution in DPBS. The sperm concentration was determined using a Thoma-Zeiss counting cell chamber (Marienfield, Germany). A total of 100 spermatozoa was evaluated at a magnification of 400x with a differential interface contrast microscope (Normarski constrast). Spermatozoa was classified as normal or abnormal. The percentage of abnormal spermatozoa was calculated. Abnormalities were referred to tail, head and middle piece and their percentage was calculated. Presence of cytoplasmatic droplets and status of the acrosome (intact or damage) in the normal spermatozoa was evaluated and their percentages were calculated.

### 2.7 Blood collection and biochemical and haematological parameters

Following the blood sampling procedure described in [18], blood samples were collected into a tube with tripotassium

ethylenediaminetetraacetic acid (K3-EDTA) at weeks 0 and 15. Haematological parameters such as white blood leukocyte count (WBC, 103/μL), percentage of lymphocytes, neutrophils, monocytes, basophils and eosinophils were determined with the haematology analyser Abacus Junior Vet (Diatron, Austria).

Blood samples were collected into a lithium heparin tube at weeks 0, 5, 10 and 15. After centrifugation at 4000 rpm for 15 min, the concentrations of total bilirubin (TBIL, μmol/L), alkaline phosphatase (ALP, U/L), albumin (ALB, g/L), alanine aminotransferase (ALT, U/L), total protein (TP, g/L), globulin (GLOB, g/L), glucose (GLU, mmol/L), creatinine (CRE, μmol/L), uric nitrogen (BUN, mmol/L), amylase (AMY, U/L), calcium (Ca+2, mmol/L), potassium (K+, mmol/L), sodium (Na+, mmol/L) and phosphorus (FOS, mmol/L) were assessed. These biochemical parameters were determined with the VETSCAN (Diatron, Austria) for Comprehensive Diagnostic Profile rotors.

### 2.8 Statistical analyses

#### 2.8.1 Survival, body weight and feed intake

Keplen Meier plot was used for the survival analyses (GrpahPad Prism 9.0.0)

Body weight and feed intake were analysed using the following model:

$$Yijkl = \mu + Wi + Dj + Wi \times Dj + mijk + eijkl;$$

Where Wi is the week effect (i=15), Dj is the diet effect (j=2; Control diet and Enriched diet); Wi x Dj is the interaction between week and diet, mijk is the random effect of the male and eijkl is the residual term. The body weight was also included as covariate for feed intake

#### 2.8.2 Seminal parameters.

The percentage of normal ejaculates was analysed using Chi-square test. Seminal parameters were analysed using the following model:

$$Yijklm = \mu + Oi + Wj + Dk + mijkl + eijklm;$$

Where Oi is the collection order effect (i=2; first and second), Wj is the week effect (j=11), Dk is the diet effect (k=2; Control diet and Enriched diet), mijkl is the random effect of the male, and eijklm is the residual term.

#### 2.8.3 Haematological and biochemical traits

Data were analysed using the following model:

$$Yijkl = \mu + Wi + Dj + Wi \times Dj + mijk + eijkl;$$

Where Wi is the week effect (i=2, week 0 and 15 for haematological traits; i=4, week 0, 5, 10 and 15 for biochemical traits), Dj is the diet effect

(j=2; Control diet and Enriched diet); Wi x Dj is the interaction effect, mijk is the random effect of the male and eijkl is the residual term.

Residuals and male effects were assumed to be independently normally distributed with the same variance. A Bayesian analysis was used, with bounded flat priors for all unknown parameters. Marginal posterior distributions were estimated for all unknowns using Gibbs sampling. Marginal posterior distributions of the differences between lines were computed with the program Rabbit developed by the Institute for Animal Science and Technology (Valencia, Spain). Monte Carlo Markov chains of 60000 iterations, with a burn-in period of 10000, and only one out of every 10 samples was saved for inferences. Convergence was tested using the Z criterion of Geweke and Monte Carlo sampling errors were computed using time-series procedures.

Results are presented with Bayesian methodology. We provide the difference between diets ($D_{D-E}$) and the precision of our estimation, finding the shortest interval with 95% probability of containing the true value, that can be asymmetric around the estimation. This is called the highest posterior density interval at 95% probability. We also calculate the actual probability of the difference between the Control diet and Enriched diet $|D_{D-E}|$ being higher than zero. We consider that there is enough evidence for the Control and Enriched diets being different when the probability of this difference in absolute value $|D_{D-E}|$ is more than 90%.

## 3. Results

### *3.1. Survival, body weight and feed intake*

Males fed with Enriched diet displayed similar survival rate to Control diet (Fig. 2a). Survival rate was 78.6 % for Enriched diet and 73.3 % for Control diet (Chi square = 0.07; P value = 79 %; data not shown in tables).

In general, body weight was 3514 g in Control diet and 3433 g in Enriched diet (P = 0.85, Table 1). Feed intake was 5% higher with the Control diet (125.2 g) than with the Enriched diet (118.6 g; P = 1.00). This difference was not due to a higher body weight of Control diet, since when the body weight was included as a covariate, the difference between diets was maintained. The evolution of the body weight and feed intake each week is shown in Figures 2b and 2c.

### *3.2. Sperm quality*

Both diets showed similar percentage of eliminated ejaculates due to low macroscopic quality (12% in the Control diet and 14% in the Enriched Diet; Chi square = 0.58; P = 45%; data not shown in tables).

Volume, motility and production were similar in both diets (Table 2). Enriched diet showed lower percentage of abnormal spermatozoa than Control diet (22 % and 30 %, respectively; P = 0.93). This difference was due to the lower percentage of tail abnormalities (16 % and 24 %, respectively; P = 0.90). Similar percentage of head and middle piece abnormalities were found in both diets (4 % and 2 %, respectively).

Similar cytoplasmatic droplet was shown for both diets (P = 0.69). Acrosome integrity was higher in Enriched than Control diet (97 % and 96 % respectively; P = 0.87).

### *3.3. Haematological and biochemical parameters*

Figures 3 and 4 show haematological parameters for diets and at the beginning and end of the experiment. WBC did not vary between diets or throughout the experiment. Lymphocytes increased 15 % and 20 % in the Control diet (P = 0.90) and in the Enriched diet (P = 0.93). Monocytes increased for the Control diet (P = 0.97) but they did not vary in the Enriched Diet. Neutrophils decreased in the Control diet (P = 0.90) and in the Enriched diet (P = 0.99). Eosinophils and basophils increased from week 0 to 15 in both Diets (P = 1.00 and P = 0.91, respectively).

Alanine aminotransferase is shown for Control and Enriched diets at 0, 5, 10 and 15 weeks in Figure 5a. Alanine aminotransferase was higher in the Control diet than in the Enriched diet at 5 week (P = 0.93) and at 10 week (P = 0.94). Both diets decreased the alanine aminotransferase, but this decrease was lower in Control diet (5.6 U/L; from 50.2 to 44.6 U/L) than in Enriched diet (6.0 U/L; from 43.5 to 37.5 U/L; P=0.95; results not shown in Figure). Alkaline phosphatase was similar for both diets and throughout the entire control period (Fig 5b). Nevertheless, while the difference between 0 and 15 weeks was similar in Control diet (39.6 and 35.5 U/L, respectively; P = 0.62), the alkaline phosphatase exhibited relevant reduction in Enriched diet (42.7 and 35.5 U/L, respectively; P = 0.97). Amylase tends to be higher in Control diet than in Enriched diet, showing difference at week 10 (P = 0.95; Figure 5c). Glucose was similar for both diets and ranged from 5.6 to 6.5 mmol/L (Fig. 5d).

Enriched diet showed a higher total protein than Control diet after the adaptation period (+ 2.68 g/L; P = 0.99; Fig. 6a) and was maintained until week 10 (+3.09 g/L; P = 0.99). However, after feeding Enriched diet for 15 weeks, the total protein was similar to Control diet. Control diet showed a lower globulin concentration than the Enriched diet in both 5 (P = 0.98; Fig. 6b) and 10 weeks (P = 0.99). Albumin was higher at the beginning of the experiment in the Control diet (22.9 g/L; Fig., 6c) than in the Enriched diet (21.9 g/L; P = 0.94). Both diets presented similar albumin from 5 to 15 week.

Control diet showed higher creatinine values than the Enriched diet (P = 0.92) at week 0, but the values were similar at week 5, 10 and 15 (Fig 7a). Both diets decreased creatinine during the experiment (-20.8 μmol/L in Control diet, P = 1.00; -30.5 μmol/L in Enriched diet, P = 1.00). Regarding uric nitrogen, similar concentration was showed for both diets (Fig. 7b) and uric nitrogen increased during the experiment (+0.7 mmol/L in both lines; P=0.99). Total bilirubin was similar in both diets (Fig. 7c) and decreased during the experiment (-0.3 μmol/L in Control diet, P = 0.92; -0.4 μmol/L in Enriched diet, P = 0.96).

The results of calcium, phosphorus, potassium and sodium are presented in Figure 8. Calcium was higher in Control diet both in 0 week (P = 0.93) and in 15 week (P = 0.97) and phosphorus was lower for the 4 (P = 0.90) and 15 weeks (P = 0.92). Potassium and sodium were similar for the two diets throughout the experimentation period.

| | Week | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Activity | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
| Adaptation period | | | | | | | | | | | | | | | | |
| Experimental period | | | | | | | | | | | | | | | | |
| Body weight, feed intake and semen extraction | | | | | | | | | | | | | | | | |
| Semen evaluation | | | | | | | | | | | | | | | | |
| Haematological parameters | | | | | | | | | | | | | | | | |
| Biochemical parameters | | | | | | | | | | | | | | | | |

265 **Figure 1**. Experimental design diagram

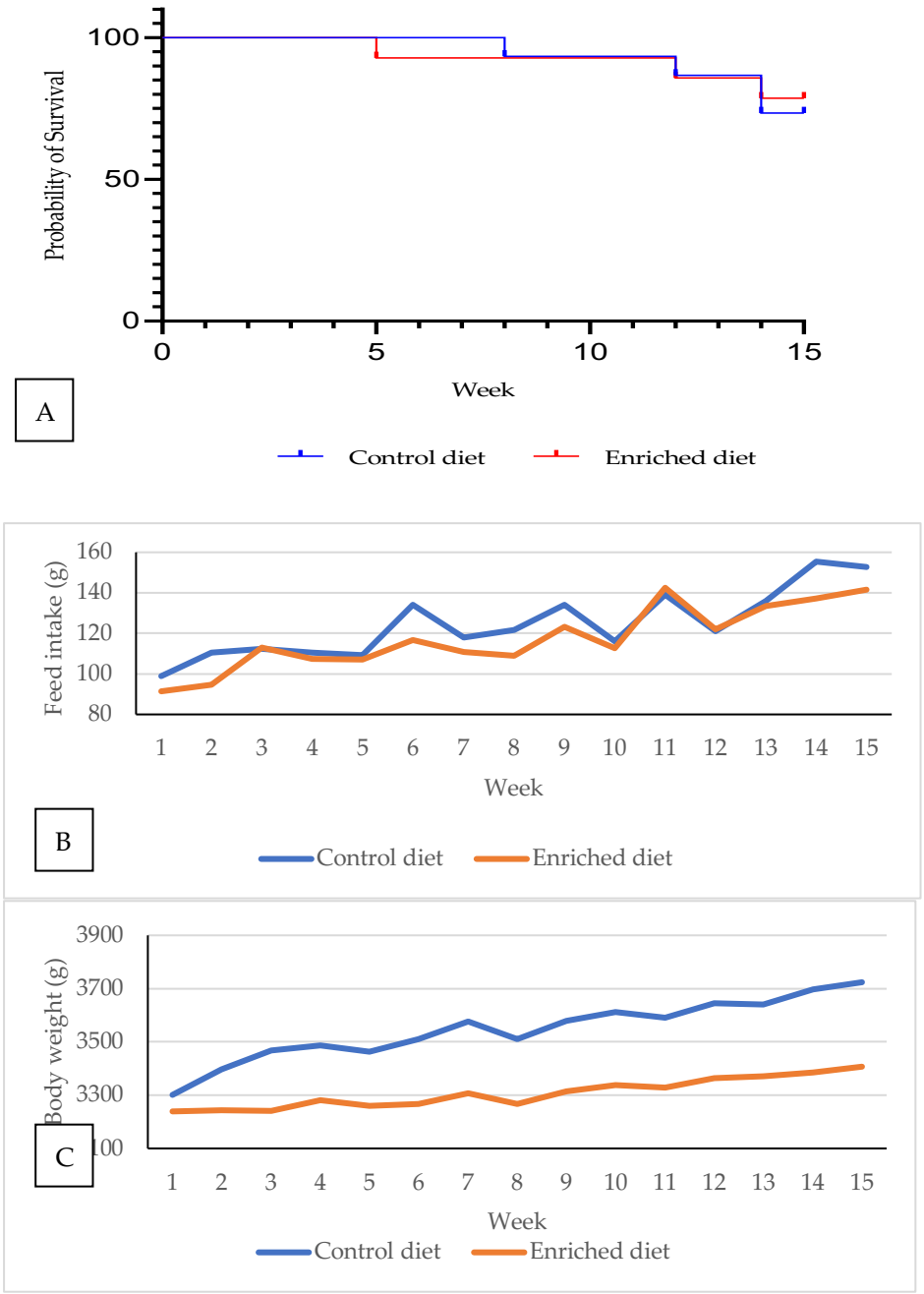



**Figure 2**. Control and Enriched diet: (A) Keplen-Meier plot. (B) Evolution of body weight. (C) Evolution of feed intake

**Table 1.** Effect of diet on body weight and feed intake in male rabbits

| | D | E | $D_{D-E}$ | $HPD_{95\%}$ | P |
|---|---|---|---|---|---|
| **Body Weight (g)** | 3514 | 3443 | 71 | -66, 202 | 0.85 |
| **Feed intake (g/day)** | 125.2 | 118.6 | 6.6 | 2.0, 10.7 | 1.00 |
| **Feed intake (g/day) *** | 125.3 | 118.3 | 7.0 | 2.7, 11.4 | 1.00 |

D: median of the Control diet; E: median of the Enriched diet; $D_{D-E}$: difference between the Control and Enriched diet; $HPD_{95\%}$: highest posterior density region at 95%; P: probability of the difference being > 0. * Body weight as covariate

**Table 2.** Effect of diet on sperm quality in male rabbits

| | D | E | $D_{D-E}$ | $HPD_{95\%}$ | P |
|---|---|---|---|---|---|
| **Volume (mL)** | 1.09 | 1.13 | 0.04 | -0.27, 0.18 | 0.64 |
| **Motility** | 3.72 | 3.75 | -0.03 | -0.07, 0.62 | 0.53 |
| **Production ($10^6$ spz)** | 266.2 | 269.1 | -3.3 | -75,7, 63.1 | 0.54 |
| **Abnormal spz (%)** | | | | | |
| **Total (%)** | 30 | 22 | 8 | -2, 18 | 0.93 |
| **Head (%)** | 4 | 4 | 0 | -3, 2 | 0.64 |
| **Tail (%)** | 24 | 16 | 8 | -5, 18 | 0.90 |
| **Middle piece (%)** | 2 | 2 | 0 | -1, 1 | 0.62 |
| **Cytoplasmatic droplet (%)** | 12 | 10 | 2 | -5, 8 | 0.69 |
| **Acrosome integrity (%)** | 96 | 97 | -1 | -3, 1 | 0.87 |

D: median of the Control diet; E: median of the Enriched diet; $D_{D-E}$: difference between the Control and Enriched diet; $HPD_{95\%}$: highest posterior density region at 95%; P: probability of the difference being > 0 when $D_{D-E} > 0$ or being < 0 when $D_{D-E} < 0$.

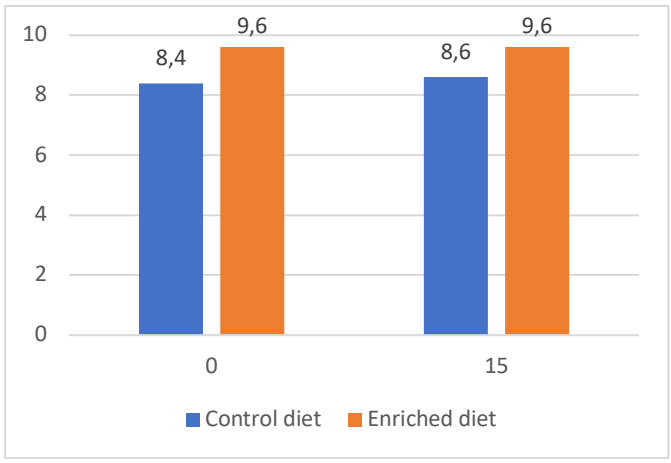



**Figure 3**. White blood cells (WBC, x10³/μL) levels for Control and Enriched diet at 0 and 15 weeks.

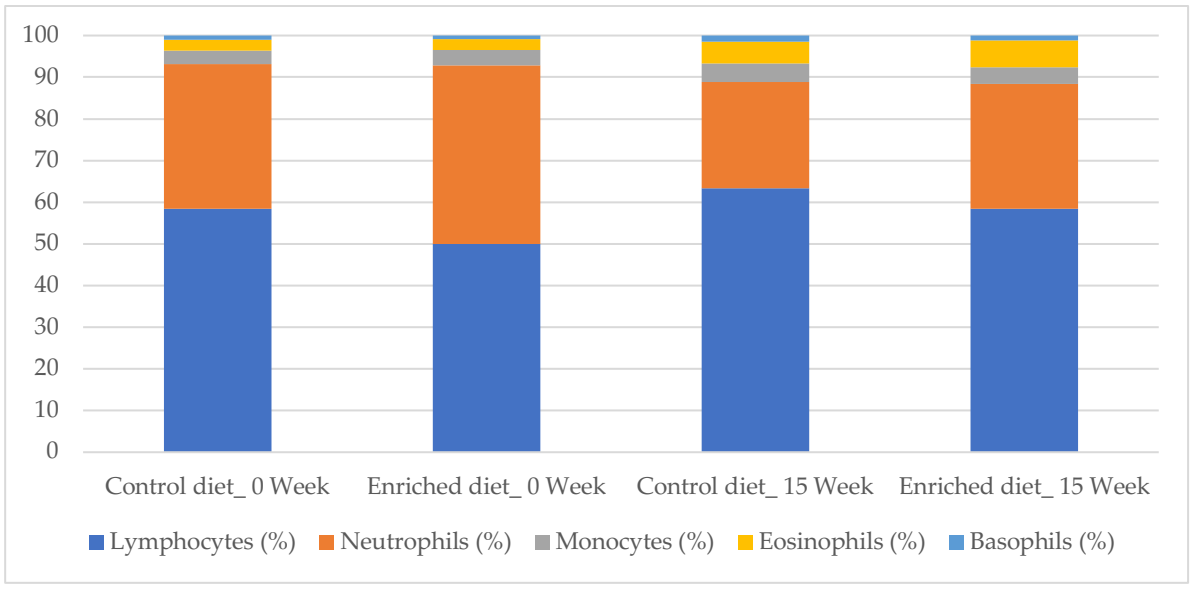



**Figure 4**. Percentage of lymphocytes, neutrophils, monocytes, eosinophils and basophils for Control and Enriched diets at 0 and 15 weeks.

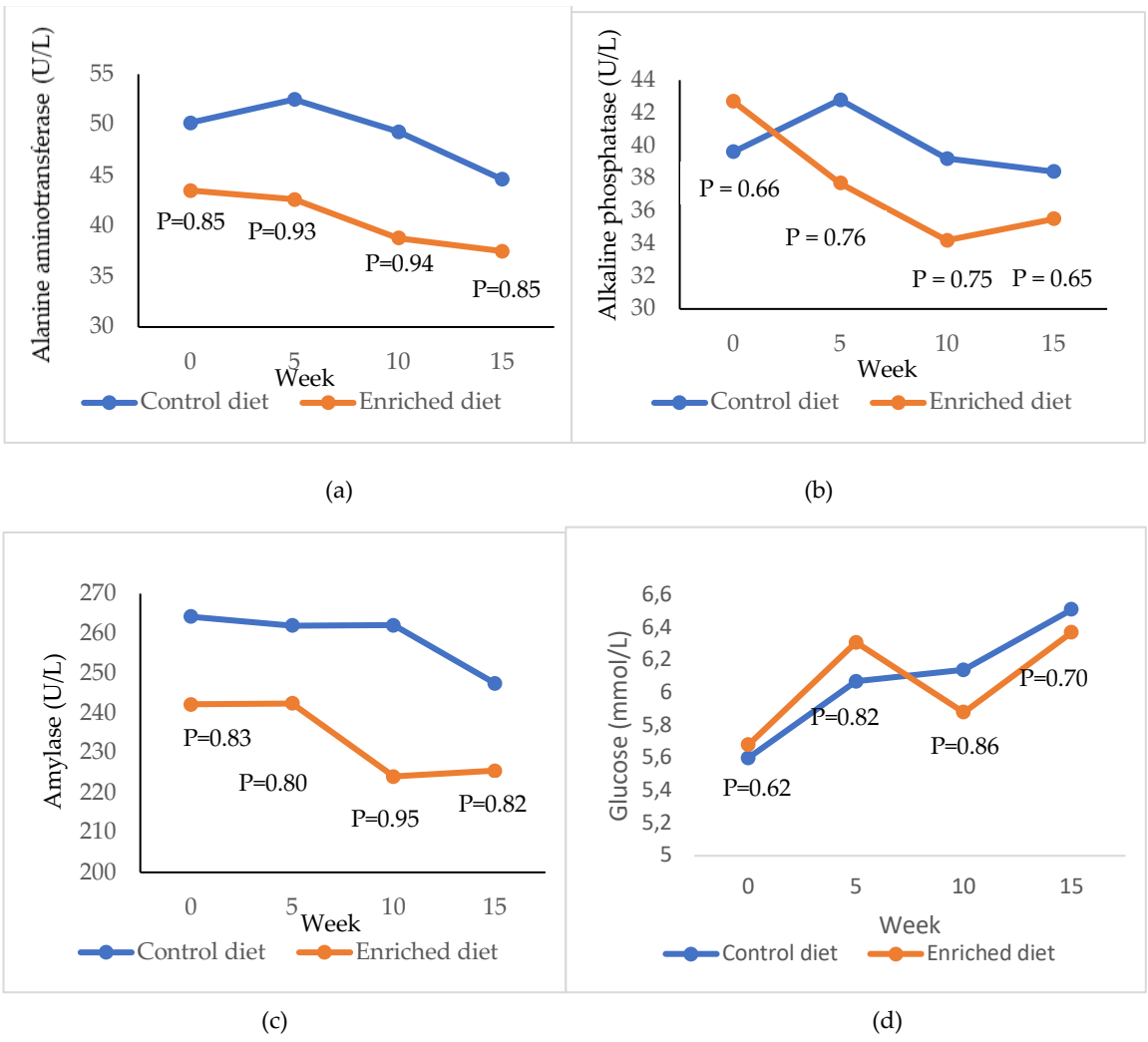
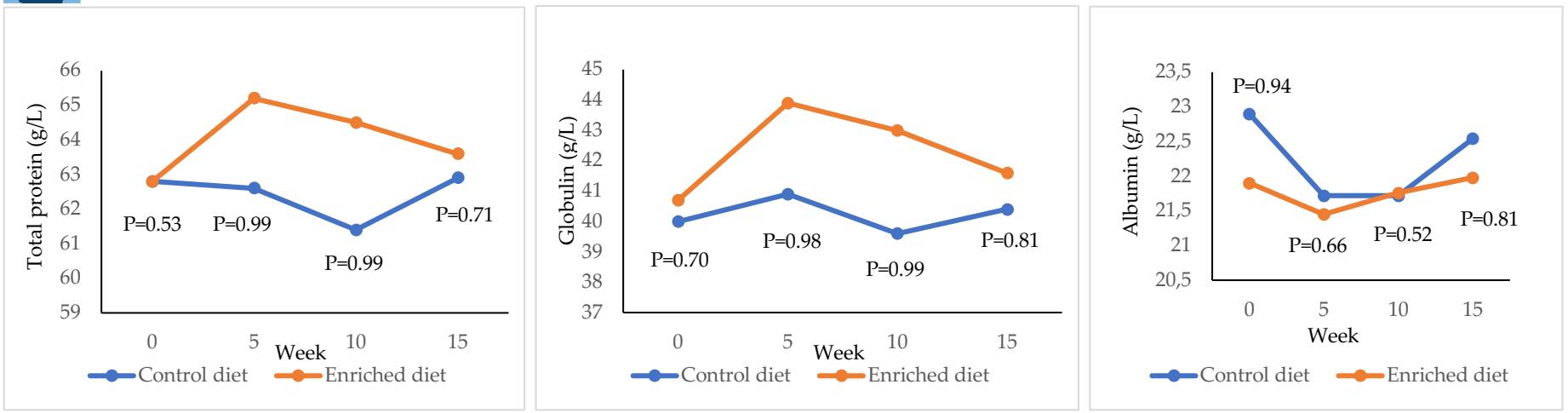



**Figure 5.** Evolution of (a) alanine aminotransferase; (b) alkaline phosphatase; (c) amylase; (d) glucose in males fed with Control and Enriched diet. P is probability of the difference being > 0 when the difference between the diets was > 0 or being < 0 when this difference was < 0.




(a) (b) (c)

**Figure 6.** Evolution of (a) total protein; (b) globulin; (c) albumin in males fed with Control and Enriched diet. P is probability of the difference being > 0 when the difference between the diets was > 0 or being < 0 when this difference was < 0.

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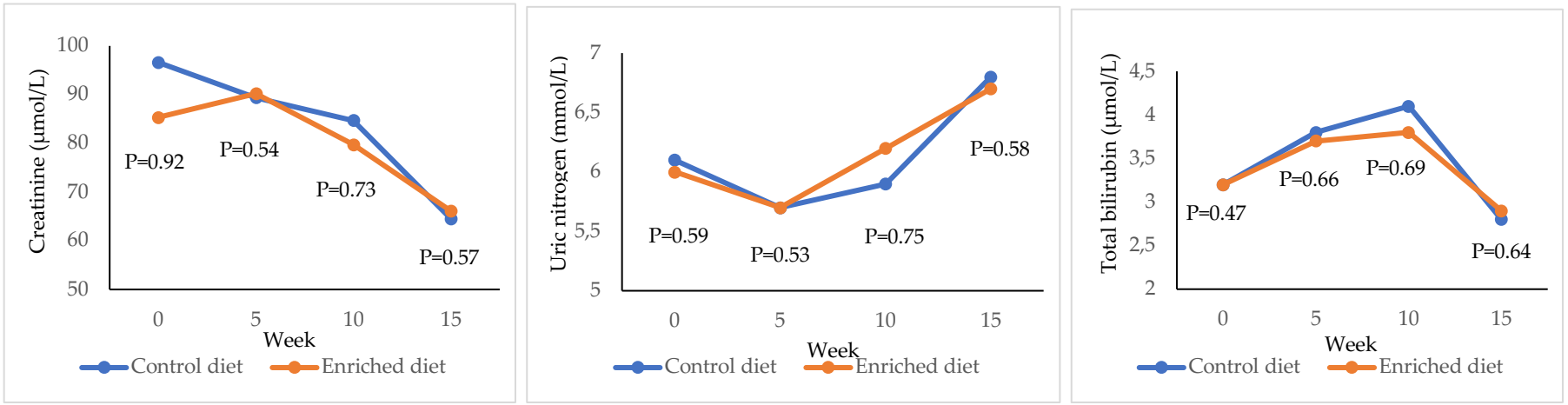


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301 (a) (b) (c)

302 **Figure 7.** Evolution of (a) creatinine; (b) uric nitrogen; (c) total bilirubin in males fed with Control and Enriched diet. P is probability of the difference being > 0 when the

303 difference between the diets was > 0 or being < 0 when this difference was < 0.

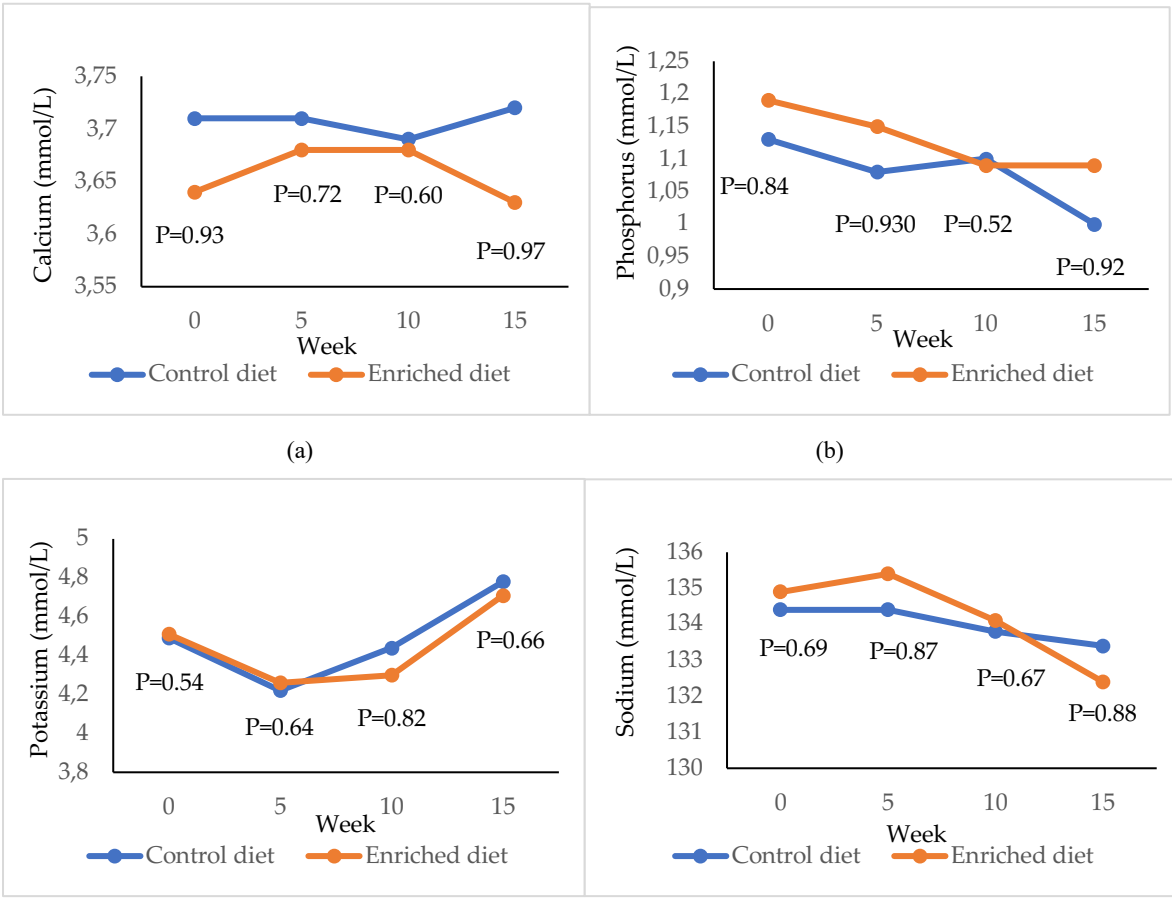


**Figure 8.** Evolution of (a) calcium; (b) phosphorous; (c) potassium; (d) Sodium in males fed with Control and Enriched diet. P is probability of the difference being > 0 when the difference between the diets was > 0 or being < 0 when this difference was < 0.

## 4. Discussion

There is an increasing evidence of the role of postbiotics as health promoter. The beneficial effects of postbiotics are mediated through an interaction between the microbial products and host [10]. In this study we have tested the effectiveness of a postbiotic formulated with a fermented food product in semen quality and health status of the male rabbit. The postbiotic has recently demonstrated the ability to improve welfare and health in diabetics rats [16] and dairy heifer calves [19,20].

Food intake is lower with the postbiotic than control diet from the second week. Nevertheless, survival was not affected. When this diet has been applied to dairy heifer calves, there has also been a decrease in consumption from week 5 of intake [19].

Many studies have been carried out to improve the seminal quality in rabbits by supplementing the feed with probiotics [21,22]. As far as we concern, no information has been found regarding postbiotics. In our experiment, a slight improvement in the acrosome integrity and spermatozoa with normal tail has been obtained in the Enriched diet, although an increase in motility has not been provided.

Hematological parameters provide valuable information on the health status of the animal. In the present study, hematological profile is within the range of healthy rabbits both at the beginning and end of the experiment and for both diets [18,23]. Levels of albumin, alkaline phosphatase, alanine aminotransferase, total bilirubin, total protein, globulin, glucose, creatinine, uric nitrogen and amylase are within the wide range of values reported in rabbits [18,24,25].

Alanine aminotransferase and alkaline phosphatase are markers of hepatic diseases [26,27] and alkaline phosphatase is also related with other disorders like increase of bones deposits, intestinal damage, hipertiroidism, and generalised tissue damage [28]. Males fed with postbiotic diet shows lower alanine aminotransferase and alkaline phosphatase concentration, thus liver profile is improving. The benefit of the postbiotic on liver function has also been demonstrated in rats [16]. Alanine aminotransferase has been decreased in meat rabbit fed with lactic acid bacteria additive [29]. Moreover, a negative correlation between these biomarkers in plasma and semen quality, mainly the motility and the acrosomal damage, has been reported in rabbits [30] and in goats [31]. As previously mentioned, the improvement in acrosome and tail would agree with this result.

Several studies have reported the hypoglycemic effect of probiotic and fermented products [32,33]. Our result indicates that amylase tend to be lower with the postbiotic. This effect is not immediate, but it occurs after consuming the diet for 10 weeks. Glucose levels were attenuated with the fermented food product in rats due to changes in the gut microbiota composition [16].

Principal plasma proteins are albumin and globulin [34]. Globulin can be considered as a good indicator of immunity response [35]. The fermented product increased a 2.5% total protein and a 5.2% globulin, whereas the albumin concentration was similar in both diets. Thus, it could be indicated that postbiotic improve immunity to infectious agents. Similar results have been obtained in calves supplemented with this postbiotic [21]. It has been found that postbiotics from *Lactobacillus plantarum* also confers anti-inflammatory responses, as observed in a study in porcine intestinal epithelial cell lines [36].

We have measured uric nitrogen and creatinine as biomarkers of kidney function status. The results indicate that kidney function has not been affected by the use of the postbiotic, since both biomarkers evolved in a similar way during the experiment for the Control and Enriched diet.

Little information is available supplementation on blood minerals in response to postbiotics. Minerals act as structural and functional cofactors in metal-containing enzyme [37]. In addition, phosphorus is part of the ATP molecule, which is the major energy source for cellular function [38]. The postbiotic increased phosphorous levels in rabbit bloods. This finding is supported by [37] in rabbits fed with probiotics and an

improvement on metabolic state of the rabbits could be expected. The results regarding calcium are not conclusive. The postbiotic equalizes the calcium levels of the animals with the Control diet, although the calcium decreases to the initial values in the last week of treatment.

## 5. Conclusions

In conclusion, postbiotics based on lactic acid bacteria improve health status of the rabbit males, especially with respect to the liver function. It also improves sperm quality, specifically the quality of the tail and the acrosome of the spermatozoid. The improvement in postbiotic intake should be investigated as it could affect the results obtained in the long term.

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**Author Contributions:** Conceptualization, M.L.G. and J.V.D.C.; formal analysis, M.L.G. and M.J.A.; writing—original draft preparation, M.L.G.; writing—review and editing, M.L.G., M.J.A., J.V.D.C. All authors have read and agreed to the published version of the manuscript..

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**Institutional Review Board Statement:** The study was conducted according to the guidelines of the Council Directives 98/58/EC and 2010/63/EU, and approved by the University Miguel Hernández of Elche Research Ethics Committee (reference number 2019/VSC/PEA/0163 approved on 5 September 2019).

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

[1] Hill, C.; Guarner, F.; Reid, G.; Gibson, G. R.; Merenstein, D. J.; Pot, B.; Morelli, L.; Canani, R.B.; Flint H.J.; Salminen, S.; Calder, P.C.; Sanders, M.A. The international scientific association for probiotics and prebiotics consensus on the scope and appropriate use of the term probiotic. *Nature Rev. Gastroenterol. Hepatol*. **2014**, 11, 506–514. doi:10.1038/nrgastro.2014.66

[2] Sarowska, J.; Choroszy-Król, I.; Regulska-Ilow, B.; Frej-Mądrzak, M.; Jama-Kmiecik, A. The therapeutic effect of probiotic bacteria on gastrointestinal diseases (Review). *Adv. Clin. Exp. Med.* **2013**, 22 (5), 759-766.

[3] Bron, P.A.; Tomita, S.; Mercenier, A.; Kleerebezem, M. Cell surface-associated compounds of probiotic lactobacilli sustain the strain-specificity dogma. *Curr. Opin. Microbiol*. **2013**, 16, 262–269. DOI: 10.1016/j.mib.2013.06.001

[4] Nader-Macías, M.E.F.; Otero, M.C.; Espeche, M.C.; Maldonado, N.C. Advances in the design of probiotic products for the prevention of major diseases in dairy cattle. *J. Ind. Microbiol. Biotechnol*. **2008**, 35, 1387–1395. https://doi.org/10.1007/s10295-008-0438-2

[5] Kelsey, A.J.; Colpoys, J.D. Effects of dietary probiotics on beef cattle performance and stress. *J. Vet. Behav*. **2018** 27, 8-14. https://doi.org/10.1016/j.jveb.2018.05.010.

[6] Dowarah, R.; Verma, A.K.; Agarwal, N. The use of Lactobacillus as an alternative of antibiotic growth promoters in pigs: A review. *Anim. Nutr.* **2017,** 3(1), 1-6. https://doi.org/10.1016/j.aninu.2016.11.002.

[7] Zhang, J.L.; Xie, Q.M.; Ji, J.; Yang, W.H.; Wu, Y.B.; Li, C.; Ma J.Y.; Bi, Y.Z. Different combinations of probiotics improve the production performance, egg quality, and immune response of layer hens. *Poult. Sci. J.* **2012**, 91(11), 2755-2760. https://doi.org/10.3382/ps.2012-02339.

[8] Bhatt, R.S.; Agrawal, A. R., Sahoo, A. (2017) Effect of probiotic supplementation on growth performance, nutrient utilization and carcass characteristics of growing Chinchilla rabbits. Journal of Applied Animal Research, **2017**, 45(1), 304-309. DOI: 10.1080/09712119.2016.1174126

[9] Aguilar-Toalá, J.E.; Garcia-Varela, R.; Garcia, H.S., Mata-Haro, V.; González-Córdova, A.F., Vallejo-Cordoba B.; Hernádez-Mendoza, A. Postbiotics: an evolving term within the functional foods field. *Trends Food. Sci. Technol*. **2018,** 75, 105–114. doi: 10.1016/j.tifs.2018.03.009

[10] Teame, T.; Wang, A.; Xie, M.; Zhang, Z.; Yang, Y.; Ding, Q.; Gao, C.; Olsen, R. E.; Ran, C.; Zhou, Z. (2020). Paraprobiotics and postbiotics of probiotic *Lactobacilli*, their positive effects on the host and action mechanisms: A Review. *Front. Nutr.* **2020**, 7, 570344. https://doi.org/10.3389/fnut.2020.570344

[11] Humam, A.M.; Loh, T.C.; Foo, H.L.; Samsudin, A.A.; Mustapha, N.M.; Zulkifli, I.; Izuddin, W.I. Effects of feeding different postbiotics produced by *Lactobacillus plantarum* on growth performance, carcass yield, intestinal morphology, gut microbiota composition, immune status, and growth gene expression in broilers under heat stress. *Animals* **2019**, 9, 644. doi: 10.3390/ani9090644

[12] Saettone, V.; Biasato, I.; Radice, E.; Schiavone, A.; Bergero, D.; Meineri, G. State-of-the-art of the nutritional alternatives to the use of antibiotics in humans and monogastric animals. *Animals* **2020**, 10, 2199. https://doi.org/10.3390/ani10122199

[13] García, M.L.; Argente, M.J. The genetic improvement in meat rabbits. In *Lagomorpha characteristics*, Ed Intechopen. **2020;** DOI: 10.5772/intechopen.93896.

[14] Catellini, C.; Dal Bosco, A.; Arias-Álvarez, M.; Lorenzo, P.L.; Cardinali, R.; Garcia Rebollar, P. The main factors affecting the reproductive performance of rabbit does: a review. *Anim. Reprod. Sci.* **2010,** 122 (3-4), 174.182. DOI: 10.1016/j.anireprosci.2010.10.003

[15] García-Tomás, M.; Sánchez, J.P.; Rafel, O.; Ramón, J.; Piles, M. Variability, repeatability and phenotypic relationships of several characteristics of production and semen quality in rabbit. *Anim. Reprod. Sci.* **2006**, 93(1-2), 88-100. doi: 10.1016/j.anireprosci.2005.06.011

[16] Cabello-Olmo, M.; Oneca, M.; Torre, P.; Sainz, N.; Moreno-Aliaga, M.J.; Guruceaga, E.; Díaz, J.V.; Encio, I.J.; Barajas, M.; Araña, M. A fermented food product containing lactic acid bacteria protects ZDF rats from the development of type 2 diabetes. *Nutrients* **2019**, 11(10), 2530. doi: 10.3390/nu11102530

[17] Argente, M.J.; García, M.L.; Zbyˇnovká, K.; Petruška, P.; Capcarová, M.; Blasco, A. Correlated response to selection for litter size environmental variability in rabbit's resilience. *Animal* **2019**, 13, 2348–2355. doi: 10.1017/S1751731119000302

[18] Beloumi, D.; Blasco, A.; Muelas, R.; Santacreu, M.A.; García, M.L.; Argente, M.J. Inflammatory correlated response in two lines of rabbit selected divergently for litter size environmental variability. *Animal* **2020**, 10, 1540. doi:10.3390/ani10091540

[19] Rovai, M.; Guifarro, L.; Anderson, J.; Salama, A. A. K. Effects of long-term postbiotic supplementation on dairy heifer calves: Performance and metabolic indicators. Abstracts of the 2019 American Dairy Science Association. Annual Meeting June 23–26, 2019 Cincinnati, Ohio. *J. Dairy Sci*. Vol. 102, Suppl. 1

[20] Rovai, M.; Guifarro, L.; Salama, A. A. K. Effects of long-term postbiotic supplementation on dairy heifer calves: Health status and wound healing after dehorning Abstracts of the 2019 American Dairy Science Association. Annual Meeting June 23–26, 2019 Cincinnati, Ohio. *J. Dairy Sci*. Vol. 102, Suppl. 1

[21] Attia, Y.A.; Ibrahim, K. Semen quality, testosterone, seminal plasma biochemical and antioxidant profiles of rabbit bucks fed diets supplemented with different concentrations of soybean lecithin. *Animal* **2012,** 6(5), 824-833. DOI: 10.1017/S1751731111002229

[22] Helal, F.I.S.; El- Badawi, A.Y.; Abou-Ward, G. A.; El-Naggar, S.; Hassan, A. A.; Basyoney, M.M.; Morad, A.A.A. Semen quality parameters of adult male NZW rabbits fed diets added with two different types of probiotics. *Egyptian J. Nutrition and Feeds* **2018**, 21 (1): 125-132

[23] Massányi, M.; Kohút, L.; Argente, M.J.; Halo, M.; Kováčik, A.; Kováčiková, E.; Ondruška, L.; Formicki, G.; Massányi, P. The effect of different sample collection methods on rabbit blood parameters. *Saudi J. Biol. Sci*. **2020**, 27(11), 3157-3160. https://doi.org/10.1016/j.sjbs.2020.07.016

[24] Leineweber, C.; Müller, E.; Marschang, R.E. Blood reference intervals for rabbits (Oryctolagus cuniculus) from routine diagnostic samples. Best immung von Blutreferenzwerten für Kaninchen (Oryctolagus cuniculus) aus Routine diagnostic proben. *Tierarztl Prax Ausg K Kleintiere Heimtiere* **2018**, 46, 393–398.

[25] Washington, I.M.; Van Hoosier, G. Clinical Biochemistry and Hematology. In The Laboratory Rabbit, Guinea Pig, Hamster, and Other Rodents; Suckow, M.A., Stevens, K.A., Wilson, R.P., Eds.; Elsevier: Amsterdam, The Netherlands, *2012*; pp. 57–116.

[26] Dontas, I.A.; Marinou, K.A.; Iliopoulos, D.; Tsantila, N.; Agrogiannis, G.; Papalois, A.; Karatzas, T. Changes of blood biochemistry in the rabbit animal model in atherosclerosis research; a time or stress-effect. *Lipids Health Dis.* **2011**, 10:139. http://www.lipidworld.com/content/10/1/139

[27] Kim,W.; Flamm, S.L.; Di Bisceglie, A.M.; Bodenheimer, H.C. Serum activity of alanine amino transferase (ALT) as an indicator of health and disease. *Hepatology* **2008**, 47, 1363–1370. doi: 10.1002/hep.22109.

[28] Fraser, C.M. *Merck Veterinary Manual*, 6th ed; Ocean: Barcelona, Spain, 2007; pp. 1314.

[29] Shah, A.A.; Liu, Z.; Chen, Q.; Juanzi, W.; Sultana, N.; Zhong, X. Potential effect of the microbial fermented feed utilization on physicochemical traits, antioxidant enzyme and trace mineral analysis in rabbit meat. *J. Anim. Physiol. Anim. Nutr.* **2019**, 104, 767–775. https ://doi.org/10.1111/jpn.13252

[30] Yousef, M.I.; Abdallah, G.A.; Kamel, K.I. Effect of ascorbic acid and Vitamin E supplementation on semen quality and biochemical parameters of male rabbits. *Anim. Reprod. Sci.* **2003**, 76, 99–111. DOI: 10.1016/s0378-4320(02)00226-9

[31] Chauhan, M.S.; Kapila, R.; Gandhi, K.K.; Anand, S.R. Acrosome damage and enzyme leakage of goat spermatozoa during dilution, cooling and freezing. *Andrologia* **1993**, 26, 21–26. https://doi.org/10.1111/j.1439-0272.1994.tb00748.x

[32] Sivamaruthi, B.S.; Kesika, P.; Prasanth, M.I.; Chaiyasut, C. A Mini Review on Antidiabetic Properties of 833 Fermented. *Nutrients* **2018**, 10, 1–18. https://doi.org/10.3390/nu11051189

[33] Ebringer, L.; Ferenčík, M.; Krajčovič, J. Beneficial Health Effects of Milk and Fermented Dairy. *Microbiol*. (Praha). **2008**, 53, 378–394. doi: 10.1007/s12223-008-0059-1

[34] Andonova, M. Role of innate defense mechanisms in acute phase response against Gram - negative agents. *Bulg. J. Vet. Med*., **2002**, 5(2), 77-92.

[35] Abdel-Azeem, A.S.; Abdel-Azim, A.M.; Darwish, A.A.; Omar, E.M. Hematological and biochemical observations in four pure breeds of rabbits and their crosses under Egyptian environmental conditions. *World Rabbit Sci*. **2010**, 18, 103-110. doi:10.4995/wrs.2010.18.13

[36] Kim, K.W.; Kang, S.S.; Woo, S. J.; Park, O. J.; Ahn, K.B.; Song, K.D.; Lee, H.J.; Yun, C.H., Han, S.H. Lipoteichoic acid of probiotic Lactobacillus plantarum attenuates Poly I:C-induced IL-8 production in porcine intestinal epithelial cells. *Front. Microbiol.* **2017**, 8, 1827. https://doi.org/10.3389/fmicb.2017.01827

[37] Shah, A.A.; Yuan, X.; Khan, R.U.; Shao, T. Effect of lactic acid bacteria-treated King grass silage on the performance traits and serum metabolites in New Zealand white rabbits (*Oryctlagus cuniculus*). *J. Anim. Physiol. Anim. Nutr*. **2018**, 102, 902–908. DOI: 10.1111/jpn.12854

[38] Jabs, C.M.; Ferrell, W.J.; Robb, H.J. Plasma Changes in Endotoxin and Anaphylactic Shock (ATP, ADP and Creatine Phosphorus). *Ann. Clin. Lab. Sci.* **1979**, 9(2), 121-132. DOI: 0091-7370/79/0300-0121.