TRADITION AND MODERNITY IN VETERINARY MEDICINE, 2021, vol. 6, No 1(10): 49–54

THE EFFECT OF RAW FOOD UNDERWENT HPP (HIGH PRESSURE PROCESSING) TREATMENT ON HAEMATOLOGY AND SERUM BIOCHEMISTRY IN DOGS

Hristina Neshovska

University of Forestry, Faculty of Veterinary Medicine, Sofia, Bulgaria
E-mail: hneshovska@abv.bg

ABSTRACT

The aim of the present study was to investigate the effect of raw food subjected to HPP technology on the haematological and biochemical parameters of the blood. The experiment included 20 clinically healthy pitbull dogs of the same weight and age, divided into three groups, which were studied in dynamics. The obtained results show an increase in Urea, ALB, and TP and a decrease in the values of Chol and ALP. No changes in haematological parameters or other biochemical parameters were detected.

Key words: raw food, dogs, haematology and serum biochemistry, HPP.

Introduction

In recent years, feeding dogs raw foods became increasingly popular among their owners (Michel, 2006). The use of raw foods led to a number of controversies, as this practice poses a potential danger to both the health of the dog and the health of the owners. (Freeman et al. 2013). Most studies related to the feeding of raw foods to dogs have focused mainly on pathogenic contamination (Lenz et al. 2009). Particular attention was also paid to the potential imbalance of nutrients in these foods, which in turn could lead to a deficit or excess of important nutrients (Freeman and Michel 2001, Freeman et al. 2013). On the other hand, in the absence of heat treatment, nutrients are preserved in raw foods, and the foods themselves had better digestibility (Hamper et al., 2015).

There are many studies showing the connection between nutrition and the health of dogs, which have shown that poor nutrition was at the root of many diseases (Sarris et al.2015, Anturaniemi et al.2020). The influence of the type of food on the blood parameters was of interest to a number of authors, who proved the direct relationship between them (Davenport et al. 1994, Swanson et al. 2004, Anturaniemi et al.2020). Haematology and serum biochemistry had important diagnostic value in determining health status, and they can be an indicator of the positive or negative impact of various factors, such as nutrition (Algya et al. 2018). With this thematic focus were the studies of Davenport et al. 1994, which investigated the effect of a protein-poor diet on some of the major biochemical parameters associated with protein metabolism. Their results showed a significant decrease in the values of Albumin, Total Protein, and Urea, as well as an increase in the concentrations of Alkaline phosphatase (Davenport et al. 1994). Khan et al. 2011 found hypoglycemia and low cholesterol levels in stray dogs, which diet was unbalanced and deficient in many nutrients.

Other authors had found a relationship between the amount of protein in the diet and the concentration of cholesterol in the blood, namely that hypercholesterolemia was observed in dogs fed high-protein diets (Kronfeld et al. 1977, Hansen et al. 1992, Swanson et al. 2004). The lack of heat treatment also affected some biochemical parameters. According to Anturaniemi et al. (2020) dogs fed a raw diet showed lower cholesterol levels than those fed a dry extruded diet. The relationship between the type of food processing and blood parameters was also observed by Algya et al. 2018, as their results showed higher values of triglycerides and alkaline phosphatase in dogs fed dry food, compared to the values of those fed raw. The authors did not find statistically significant differences in the haematology of the respective groups of dogs.

As already mentioned, one of the major risks associated with the consumption of raw food was precisely their safety in terms of microbiological contamination. Reduction of pathogen contamination could be achieved through a variety of technological methods (Delmore et al. 1998). High pressure processing (HPP) was a type of non-heat processing that eliminated pathogenic microorganisms, increased the shelf life without affecting the quality and nutritional value of the product, which was often observed as an effect of heat treatment (Bermúdez-Aguirre и Barbosa-Cánovas 2011;). According to Koutchma (2014), HPP technology was widely used in the food industry, as it obtained an all-natural and safe end product. Scientific data on the application of this technology in the production of pet food as a way of decontamination have not been found.

Materials and methods

For the present study, we used a total of 20 pitbull dogs, 10 females and 10 males. The animals were equalized in age, 3.5 ± 0.5 years and body weight 21.04 ± 1.39 kg. All of them were vaccinated and regularly dewormed for internal and external parasites, nevertheless the faeces of each of them were examined by the Füleborn method for the presence of nematodes and cestodes. Prior to the start of the study, the health status was taken from all dogs and found to be clinically healthy. During the experiments, the dogs were bred under the same environmental conditions, in strict compliance with all hygienic parameters. Feeding was performed twice a day and water was allowed ad libitum. The technical manipulations (blood sampling, clinical examinations) were performed in accordance with good clinical practice and Ordinance № 20 of 01.11.2012 on the minimum requirements for protection and welfare of experimental animals and the requirements for sites for use, breeding and/or their delivery.

Venous blood was collected from vena cephalica and vena saphena lateralis, via a closed system with vacuum containers in 2 ml EDTA K_2 and 5 ml serum tubes with clot actovator. EDTA K_2 samples were turned around 5 times instantly, placed on a tripod in a cooler bag, and transported to the laboratory. Serum tubes were centrifuged shortly after collection, after that the serum was separated into Eppendorf tubes and again transported to the laboratory via a cooler bag. We studied a standard set of hematological parameters (total number of leukocytes - WBC, number of lymphocytes - LYM, number of granulocytes - GRA, the total number of erythrocytes - RBC, hemoglobin - HGB, hematocrit - HCT, the total number of platelets - PLT and some related to the metabolism of proteins, lipids and carbohydrates (glucose - GLU, urea - UREA, creatinine - Creat, aspartate aminotransferase - ASAT, alanine aminotransferase - ALAT, alkaline phosphatase - ALP, albumin - ALB, T protein - total protein triglycerides - Triglyc), and serum electrolytes - calcium - Ca and phosphorus - P. Hematology was performed with whole blood using a device BC - 2800 Vet, MINDRAY, China, and biochemical parameters were examined in blood serum using an automatic biochemical analyzer BS - 200, MINDRAY, China.

Blood samples were taken in dynamics: at the start of the test on day 0, on the 15th, and on the 45th day from the beginning of the raw food feeding. Until the first blood draw (day 0), all dogs were fed low-class industrially produced dry extruded food.

During the experiment, the dogs were fed raw food that underwent HPP processing, based on chicken meat, and the recipe included only raw materials suitable for human consumption. Food processing was performed with "AVURE AV-20M high-pressure processing equipment" for microbiological reduction of food, with the following parameters cycle time for 3 minutes and a pressure of 6000 bar, stored at 0–4°C.

Statistical analysis

The data were analyzed using Microsoft Excel for Windows. Confidence between groups was calculated by Student T-test at $P < 0.05$.

Results and discussion

The data from the biochemical and hematological parameters of the blood in dogs for the whole period of the experiment, fed with dry and raw food, were presented in tables.

Table 1 showed the hematology in dynamics, with each indicator given separately for male and female animals. The obtained results showed statistically insignificant differences in the blood parameters during the different periods, as the statistical processing did not reject the null hypothesis at a significance level <0.05. All results were normal compared to the reference levels for dogs. Although in both sexes each indicator was within the reference values, it was noteworthy that there was a difference in the values of some hematological parameters. WBC, Lymph, Gran, and PLT had higher values in females than males, and Mon, RBC, HGB, HCT were higher in males. For the whole period of the experience, this tendency was preserved.

Table 1: Mean values of haematology for male and female dogs.

Group / Parameters	Day 0		Day 15		Day 45	
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
	Male	Female	Male	Female	Male	Female
WBC(x10^9/L)	12.65±1.72	14.19±3.89	13.73±3.23	15.89±3.45	13.46±1.17	14.14±1.89
Lymph#(x10^9/L)	3.25±1.40	3.88±1.13	2.92±0.58	3.65±1.56	3.5±0.79	4.02±1.15
Mon#(x10^9/L)	0.67±0.38	0.61±0.13	0.57±0.10	0.65±0.15	0.67±0.12	0.71±0.13
Gran#(x10^9/L)	8.4±1.93	9.72±3.35	10.24±2.87	11.59±3.30	9.34±0.85	9.73±2.36
RBC(x10^12/L)	8.30±2.176	7.23±1.68	7.44±0.93	6.93±0.58	8.27±1.071	7.50±0.44
HGB (g/L)	179.9±61.57	154.5±41.39	158.8±25.75	150.1±14.72	179.9±27.78	166.4±12.25
HCT %	55.14±14.01	49.75±11.64	49.17±7.11	47.07±3.81	54.76±7.718	50.77±3.63
PLT(x10^9/L)	214.8±81.07	288.9±103.68	267.2±41.45	358.7±123.42	246.6±60.36	312.9±89.50

Table 2 presented summary mean values for both sexes of dogs for each feeding period. Data showed that when feeding dogs raw food for a period of 45 days, no statistically significant changes in hematological parameters were observed and they remained within the reference range. Our result confirmed these obtained by Algya et al. (2018), who also did not establish significant differences in the complete blood count (CBC).

Table 3 showed the average values of the serum biochemistry in dynamics (day 0, 15^{th}, and 45^{th}), and again the dogs were divided by sex. Table 4 summarized the blood biochemistry of male and female animals. In the raw food fed group, there were an increase in total protein (TP) values from 45.87 ± 13.89 (g/l) to 57.20 ± 10.17 (g/l) on day 15 of the first intake, and 62.58 ± 4.91 g/l in the group on day 45. At days 15 and 45, mean TP values in males and females, as well as in all dogs, showed statistical significance at a significance level <0.05. According to Davenport et al. In 1994, there was a decline in some indicators related to protein metabolisms, such as albumin and urea, when feeding a low-protein diet. The dogs in our study were fed a high-protein raw food, as a result of which we found a statistically significant increase in urea and albumin for both periods (15^{th} and

45th day). Despite the higher values of UREA (4.81 ± 0.90 mmol/l) and ALB (27.13 ± 2.72 g/l), the indicators remained within the reference range at day 45.

Table 2: Mean values of haematology for all groups.

Parameters \ Group	Day 0	Day 15	Day 45
WBC (x10^9/L)	13.42±3.03	14.81±3.44	13.8±1.57
Lymph (x10^9/L)	3.56±1.28	3.28±1.20	3.76±1.00
Mon# (x10^9/L)	0.64±0.27	0.61±0.13	0.69±0.12
Gran# (x10^9/L)	9.06±2.75	10.91±3.09	9.53±1.74
RBC (x10^12/L)	7.77±1.97	7.19±0.80	7.89±0.89
HGB (g/L)	167.2±52.69	154.45±20.89	173.15±22.01
HCT %	52.445±12.84	48.12±5.66	52.76±6.21
PLT (x10^9/L)	251.85±98.23	312.95±101.15	279.75±81.71

Table 3: Mean values of biochemistry for male and female dogs.

Parameters \ Group	Day 0		Day 15		Day 45	
	Mean ± SD Male	Mean ± SD Female	Mean ± SD Male	Mean ± SD Female	Mean ± SD Male	Mean ± SD Female
GLU (mmol/l)	2.99 ±0.65	2.8±0.55	3.02±0.64	3.01±0.57	2.84±0.55	2.85±0.43
UREA (mmol/l)	2.66 ±0.29	2.54±0.44	3.12±0.44	3.35±1.12	4.96±1.09	4.66±0.69
CREAT (μmol/l)	69.66±10.19	69.19±7.90	71.47±13.92	67.77±9.03	76.5±25.70	75.27±8.27
ASAT (U/l)	35.1±8.70	39.51±7.38	54.95±11.74	42.84±10.05	45.09±10.50	49.07±20.17
ALAT U/l)	42.68±17.29	38.34±17.85	51.89±25.86	37.58±15.71	53.67±26.15	50.23±22.39
ALP (U/l)	70.45±18.23	79.35±28.34	22.95±12.41	22.02±11.57	22.7±9.18	27.6±20.70
ALB (g/l)	24.2±3.19	23.87±2.50	27.41±2.97	26.11±2.91	27.37±3.77	26.9±1.13
TP (g/l)	41.34±18.36	50.41±4.94	57.6±11.06	56.81±9.79	63.61±4.64	61.55±5.20
Chol (mmol/l)	6.29 ±1.15	5.93±0.68	5.843±1.34	5.78±0.59	5.48±0.79	5.11±0.92
Triglyc (mmol/l)	0.94 ±0.16	1.01±0.12	1.00±0.43	1.21±1.21	0.93±0.13	0.90±0.27
Ca (mmol/l)	2.382 ±0.13	2.397±0.17	2.427±0.23	2.378±0.14	2.426±0.27	2.42±0.17
P (mmol/l)	1.33 ±0.18	1.32±0.13	1.29±0.25	1.38±0.15	1.19±0.20	1.38±0.18

Table 4: Mean values of biochemistry for all groups.

Parameters \ Group	Day 0	Day 15	Day 45
GLU (mmol/l)	2.89±0.59	3.015±0.59	2.84±0.48
UREA (mmol/l)	2.6±0.36	3.23±0.84	4.81±0.90
CREAT (μmol/l)	69.42±8.88	69.62±11.57	75.88±18.59
ASAT (U/l)	37.30±8.17	48.89±12.32	47.08±15.78
ALAT (U/l)	40.76±16.96	44.73±22.08	51.95±23.76
ALP (U/l)	74.9±23.63	22.48±11.69	25.15±15.78
ALB (g/l)	24.03±2.80	26.76±2.94	27.13±2.72
TP (g/l)	45.87±13.89	57.20±10.17	62.58±4.91
Chol (mmol/l)	6.11±0.94	5.81±0.94	5.29±0.86
Triglyc (mmol/l)	0.97±0.14	1.11±0.51	0.91±0.21
Ca (mmol/l)	2.38±0.14	2.40±0.19	2.44±0.22
P (mmol/l)	1.33±0.16	1.33±0.21	1.28±0.21

A change in cholesterol levels was also observed, expressed by hypercholesterolaemia (6.11 ± 0.94 mmol/l) in dogs fed dry extruded food (day 0). On day 45 of the experiment, cholesterol values were 5.29 ± 0.86 mmol/l or 13.5% lower than day 0. Statistically significant reduction of cholesterol with prolonged feeding (45 days) with food without heat treatment, we observed in both males and females. A similar relationship between cholesterol and raw food intake in dogs has been suggested by other authors Anturaniemi et al. (2020).

The data also showed that there was a tendency for a progressive decrease in alkaline phosphatase, as early as the 15_{th} day of the raw food diet. However, transferases showed close values without significant differences throughout the experimental period. Similar results were observed for glucose, with statistically insignificant differences. Mild hypoglycaemia proved in all three blood draws, which can be explained by the fact that the last meal was in the late afternoon of the previous day. No statistically significant changes were found in the studied serum levels of calcium and phosphorus.

The presented data on the blood biochemistry showed that when feeding dogs raw food, changes of statistical significance are observed in some of the studied parameters. Despite the short feeding period, we observed a positive effect of raw food on biochemical parameters.

Conclusion

No statistically significant changes in blood haematology were observed when feeding dogs with raw food that had undergone HPP treatment for a period of 45 days.

From the serum biochemistry of dogs fed raw food, statistically, significant changes in the values of Urea, ALB, TP, Chol, Triglyc, ALP were found.

Feeding dogs raw food for a period of 45 days gave positive results on protein and lipid metabolism.

References

1. Algya K. M., Cross T. L., Leuck K. N., Kastner M. K., Baba T., Lye L., de Godoy M. K., Swanson K. S. (2018). *Apparent total tract macronu-trient digestibility, serum chemistry, urinalysis, and fecal character-istics, metabolites and microbiota of adult dogs fed extruded, mildly cooked, and raw diets*. J Anim Sci.; 96:3670–3683.
2. Anturaniemi J., Zaldivar-Lopez S., Moore R., Kosola M., Sankari S., Barrouin-Melo S., and Hielm-Bjorkman A. (2020). *The effect of a raw vs dry diet on serum biochemical, hematologic, blood iron, B12, and folate levels in Staffordshire Bull Terriers*. Vet Clin Pathol.;49:258–269. https://doi.org/10.1111/vcp.12852.
3. Bermudez-Aguirre D., Barbosa-Canovas G.V. (2011). *An update on high hydrostatic pressure, from the laboratory to industrial applications*. Food Eng. Rev. 3, 44–61. https://doi.org/10.1007/s12393-010-9030-4.
4. Davenport D. J., Mostardi R. A., Richardson D. C., Gross K. L., Greene K. A., Blair K. (1994). *Protein-deficient diet alters serum alkaline phosphatase, bile acids, proteins and urea nitrogen in dogs*. J Nutr, 124: 2677S–2679S.
5. Delmore L. G., Sofos J., Schmidt G. and Smith G. (1998). *Decontamination of inoculated beef with sequential spraying treatments*. Journal of food science 63: 890–900. https://doi.org/10.1111/j.1365-2621.1998.tb17921.x.
6. Freeman L. M. and Michel K. E. (2001). *Evaluation of raw food diets for dogs*. Journal of the American Veterinary Medical Association, Vol. 218, No. 5, Pages 705–709, https://doi.org/10.2460/javma.2001.218.705.
7. Freeman L. M., Chandler M. L., Hamper B. A. et al. (2013) *Current knowledge about the risks and benefits of raw meat-based diets for dogs and cats*. Journal of the American Veterinary Medical Association, Vol. 243, No. 11, Pages 1549–1558, https://doi.org/10.2460/javma.243.11.1549.

8. Hamper B. A., Kirk C. A., and Bartges J. W. (2015). *Apparent nutrient digestibility of two raw diets in domestic kittens*. Journal of Feline Medicine and Surgery, volume 18, issue 12, pages 991–996. https://doi.org/10.1177/1098612X15605535.
9. Hansen B., DiBartola S. P., Chew D. J., Brownie C., Nagode L. (1992). *Clinical and metabolic findings in dogs with chronic renal failure fed two diets*. Am J Vet Res. 53:326–334.
10. Khan S. A., Epstein J. H., Olival K. J., Hassan M. M., Hossain M. B., Rahman K. B., Elahi M. F., Mamun M. A., Haider N., Yasin G., Desmond J. (2011). *Hematology and serum chemistry reference values of stray dogs in Bangladesh*. Open Vet J, 1: 13–20.
11. Koutchma T. (2014). *Adapting High Hydrostatic Pressure (HPP) for Food Processing Operations 1st Edition*. Book. ISBN: 9780124200913.
12. Kronfeld D. S., Hammel E. P., Ramberg Jr C. F., Dunlap Jr H. L. (1977). *Hematological and metabolic responses to training in racing sled dogs fed diets containing medium, low, or zero carbohydrate*. Am J Clin Nutr.;30:419–430.
13. Lenz J., Joffe D., Kauffman M., Zhang Y. and LeJeune J. (2009). *Perceptions, practices, and consequences associated with foodborne pathogens and the feeding of raw meat to dogs*. The Canadian veterinary journal La revue veterinaire canadienne, 50(6), 637–643.
14. Michel K. E. (2006). *Unconventional diets for dogs and cats. The Veterinary Clinics of North America*. Small Animal Practice.Volume 36, Issue 6, pages 1269-81, vi-vii. DOI: 10.1016/j.cvsm.2006.08.003.
15. Sarris J., Logan A. C., Akbaraly T. N., Amminger G. P., Balanza-Martinez V., Freeman M. P., Hibbeln J., Matsuoka Y., Mischoulon D., Mizoue T., Nanri A., Nishi D., Ramsey D., Rucklidge J. J., Sanchez-Villegas A., Scholey A., Su K. P., Jacka F. N. (2015). *International Society for Nutritional Psychiatry Research. Nutritional medicine as mainstream in psychiatry*. Lancet Psychiatry. Mar;2(3):271–4. doi: 10.1016/S2215-0366(14)00051-0. PMID: 26359904.
16. Swanson K. S., Kuzmuk K. N., Schook L. B., Fahey G. C. Jr. (2004). *Diet affects nutrient digestibility, hematology, and serum chemistry of senior and weanling dogs*. J Anim Sci, 82: 1713–1724.