Received: 15 October 2020 | Accepted: 13 June 2021

DOI: 10.1111/jpn.13601

ORIGINAL ARTICLE

Journal of Animal Physiology and Animal Nutrition WILEY

Computer-aided ration calculation (Diet Check Munich©) versus blood profile in raw fed privately owned dogs

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Funding information
WOA Institution: LUDWIG-MAXIMILIANS-UNIVERSITAET MUNCHEN; Blended DEAL: Projekt DEAL

Abstract

Many dog owners create nutritionally imbalanced raw meat-based diets (RMBD) with information from the Internet and pseudo-scientific books, some even use pre-prepared frozen raw feed from online shops, local butchers or other providers. The risk of nutritional imbalances is therefore present. Blood profiles for dogs fed RMBD are promoted by laboratories as a simple tool for the owner to check the nutritional supply situation. Veterinarian nutrition specialists seem to be consulted less frequently and, in most cases, when blood analyses show deviations from reference ranges. The aim of the present study was to evaluate whether a RMBD blood profile reflects possible malnutrition according to a computer-aided ration check and to assess its clinical relevance. Using standardized questionnaires, the average daily rations of 104 dogs, 83 of which were fed raw diets versus 21 commercially fed dogs, were analysed using Diet Check Munich©, based on the National Research Council values. Afterwards, the SYNLAB.vet GmbH '*Barfer-Profil*' test including calcium, phosphate, calcium/phosphate ratio, vitamin A, vitamin D, copper, zinc and iodine with additional parameters taurine, urea, uric acid and creatinine was carried out. No significant correlation between nutrient supply and associated blood parameters could be detected. Diet calculation revealed significantly more nutritional imbalances in the RMBD group than in the control group. Low plasma taurine could be detected only in the RMBD group. After participating, 30% of the dog owners (RMBD group) decided to adjust their dogs' diets at the nutrition consultation of the Clinic for Small Animal Internal Medicine of the LMU Munich. Based on these results, for most parameters a RMBD blood profile is not an appropriate tool to monitor a dog's nutrition and computer-aided ration calculation remains the gold standard for detecting nutritional imbalances.

KEYWORDS
barf, nutrition, nutritional assessment, raw diet, self-created diet, trace elements

1 | INTRODUCTION

For more than a decade raw meat-based diets (RMBD) have been enjoying an increase in popularity amongst dog owners. They have developed into their own feeding method with many followers. In this kind of feeding, commercially available complete dry or wet food and cereals are completely or largely avoided as a part of the main diet and instead, raw meat, bones, oils/fats, fruit, and vegetables are given to the dog (Dillitzer et al., 2012; Kamphues, 2014; Kölle & Schmidt, 2015).

The supposed main argument for this special form of nutrition is that only raw meat and animal by-products are considered suitable for dogs, and that only this can be considered healthy. The dog ought to be fed similar to the natural diet of the wolf in nature. In addition, it is assumed that the possibly high cereal content in commercial dog food could have an adverse effect on the health of the dog, since it is supposedly not part of the dog's natural diet (Kamphues, 2014; Kölle & Schmidt, 2015). According to dog owners, other advantages of RMBDs versus commercial complete diets/foods are as follows: 1) extended feeding times and thus satisfying chewing needs, provided the food is not minced too much, 2) a precise knowledge of what is being fed, provided no ready-to-eat packages are fed and 3) regular bone chewing can positively influence dental health (Dillitzer et al., 2012; Vervuert & Rückert, 2017). In addition, in case of special dietary requirements, such as for certain conditions, preferences and aversions, an individual and needs-based diet can be provided (Dillitzer et al., 2012; Zentek, 2016a).

Nevertheless, one of the main problems with diets individually created by dog owners is nutrient imbalances. If an individually created diet is used over several weeks as a normal diet, a calculational ration check is absolutely necessary (Dillitzer et al., 2012; Kamphues, 2014; Stockman et al., 2013).

The study of Dillitzer et al., (2011) examined raw food rations of adult dogs for nutritional imbalances using a computer-aided ration calculation program. Results showed that 25% of all rations contained only 70% or less of the recommended daily allowance for vitamin A. Only 25% of the recommendation for calcium (Ca) was fed in 10% of the rations. In addition, the Ca to phosphorus (P) ratio in these rations was below 0.6/1 and there was clearly too little vitamin D provided. In about half of the rations examined, the minimum requirement for iodine (I) according to the National Research Council (NRC) was not met. More than half provided less zinc (Zn) and copper (Cu) than the recommendation. Overall, 60% of the rations showed at least one or more imbalances. Another study of Stockman et al., (2013) showed that 95% of all RMBD recipes studied were found to contain at least one or more essential nutrients below the Association of American Feed Control Officials (AAFCO) minimum. The study also revealed multiple nutritional imbalances in 84% of the rations.

In contrast, deficiencies or excesses in the daily diets can be reliably identified by calculation of the current supply level and adjusted by comparison with the individual nutritional requirements based on the NRC (Kölle & Schmidt, 2015; Thes & Dillitzer, 2014). When a diet is created or checked using a computer-based program based on scientific data, all the components are present in the appropriate amounts. The nutrients contained in the daily amount of each component are summed and then compared with the individual requirements of the dog depending on body weight, age, sex and activity level (Dillitzer & Fritz, 2009).

The main objective of this study is to evaluate whether an analysis of certain blood parameters according to the commercially available screening profile, in this case, '*Barfer-Profil Hund*' by SYNLAB.vet GmbH, Augsburg, Germany (2020), can be used to monitor nutritional imbalances in the diet. Furthermore, it should demonstrate to which extend and how a clinical relevance of such screenings could be given and its correlation, compared to the calculated ration control. Finally, we want to draw conclusions as to whether the screening test could be improved by adding several promising parameters used to detect kidney diseases that have been empirically observed in RMBD-fed dogs by the nutrition consultation of the Clinic of Small Animal Internal Medicine of the LMU Munich.

2 | MATERIAL AND METHODS

The inclusion criteria for the current study were as follows: (a) male/female, intact/neutered dog older than 1 year, (b) fed raw (RMBD group)/fed with complete commercial food (control group) for at least 1/2 year, (c) the fed diet must be fully calculable prior to blood collection, and (d) to be clinically healthy. A total of 104 adult dogs aged between 1 and 13 years met the inclusion criteria for this study. Of these dogs, 83 were fed a RMBD and 21 were fed a commercially available dry or wet feed acting as a control group. In the RMBD group, 35 dogs were male, 21 of which were neutered and 48 were female, 25 of which were neutered. In the control group were 15 male dogs, 8 of which were neutered and 6 female dogs, 5 of which were neutered. The largest breed groups were mixed-breeds (n = 21 dogs) in the RMBD group and Labrador Retrievers (n = 6 dogs) in the control group. A total of 11 dogs were reported to be allergic according to their owners (10 in the RMBD group, 1 in the control group).

The average daily rations of all 104 dogs were evaluated using a standardized questionnaire. These rations were then calculated by the computer-aided calculation program Diet Check Munich© (Dobenecker & Kienzle, 2017) for the daily average nutrient content of the diets. Nutritional information for the commercial complete feeds was also available in Diet Check Munich©, provided directly from the manufacturer, or used from already existing information according to the declaration. A focus was put on the following parameters: energy intake, energy density, crude protein, Ca, P, Ca/P ratio, vitamin A and D, Cu, I and Zn. Last but not least the calculated average nutrient contents of each diet was compared with the individual recommended nutrient level for adult dogs per $kg^{0.75}$ (RNL) according to FEDIAF (2020) and the recommended allowance per $kg^{0.75}$ (RA) according to NRC (2006). Following this, nutrients

TABLE 1 Comparison of raw meat-based diets (RMBD) and control group diets on the amount (%) of respective nutrients contained. Categorized in meeting the recommended nutrient levels (RNL) per $kg^{0.75}$ (FEDIAF, 2020), exceeding the nutritional maximum (max) per kg dry matter (DM) of feed (FEDIAF, 2020) or the safe upper limit (SUL) per kg DM (NRC, 2006), and surpassing RNL (FEDIAF, 2020)

	Meeting RNL		Below RNL		Exceeding max or SUL		
	RMBD[a]	Control[b]	RMBD[a]	Control[b]	RMBD[a]	Control[b]	
Crude protein	97.6%	90.5%	2.4%	9.5%	–	–	n/a
Calcium	68.7%	100.0%	19.3%	0.0%	12.0%	0.0%	max
Phosphorus	75.9%	100.0%	10.8%	0.0%	13.3%	0.0%	max
Ca/P Ratio	72.3%	100.0%	19.3%	0.0%	8.4%	0.0%	max / SUL
Iodine	29.0%	95.2%	60.2%	0.0%	10.8%	4.8%	max
Copper	36.1%	100.0%	63.9%	0.0%	–	–	n/a
Zinc	24.1%	85.7%	75.9%	14.3%	–	–	n/a
Vitamin A	90.3%	100.0%	9.7%	0.0%	0.0%	0.0%	max / SUL
Vitamin D	36.1%	90.5%	63.9%	9.5%	0.0%	0.0%	max / SUL

[a]n = 83.
[b]n = 21.

TABLE 2 Levels (mean ± standard deviation (minimum–maximum)) of nutrients calculated in the raw meat-based diet (RMBD) group and the control group and their respective recommended allowance (RA) according NRC (2006) and recommended nutrient level (RNL) according to FEDIAF (2020)

	Mean nutrient supply			RA (NRC, 2006)	RNL (FEDIAF, 2020)
	RMBD group (n = 83)	Control group (n = 21)	*p*-value		
Crude protein (g/ $kg^{0.75}$)	10.90 ± 4.15 (4.78–25.14)	6.70 ± 1.78 (4.41–11.22)	**<0.001**	3.28	4.95
Calcium (g/$kg^{0.75}$)	0.37 ± 0.50 (0.02–4.17)	0.31 ± 0.10 (0.15–0.55)	0.108	0.13	0.14
Phosphorus (g/$kg^{0.75}$)	0.24 ± 0.28 (0.07–2.44)	0.23 ± 0.07 (0.13–0.40)	0.061	0.10	0.11
Ca/P Ratio	1.37 ± 0.45 (0.20–2.80)	1.37 ± 0.18 (1.20–1.70)	0.736	1.30	1.27[a]
Iodine (μg/$kg^{0.75}$)	56.06 ± 140.45 (0.00–956.17)	50.06 ± 17.22 (31.28–94.60)	**<0.001**	29.60	30.00
Copper (mg/$kg^{0.75}$)	0.19 ± 0.18 (0.00–1.55)	0.38 ± 0.28 (0.12–1.18)	**<0.001**	0.20	
Zinc (mg/$kg^{0.75}$)	1.81 ± 1.85 (0.50–13.42)	3.94 ± 1.67 (1.71–7.43)	**<0.001**	2.00	
Vitamin A (IU/$kg^{0.75}$)	676.69 ± 527.06 (0.42–2690.90)	391.40 ± 248.48 (177.18–1161.92)	**0.016**	167.00	
Vitamin D (IU/$kg^{0.75}$)	16.12 ± 19.17 (0.19–114.01)	26.36 ± 8.40 (13.12–42.76)	**<0.001**	18.00	15.20
Energy (kcal/kg DM)	4750 ± 441 (3278–5886)	4073 ± 429 (3432–5092)	**<0.001**		

[a]Calculated value according the requirements of Ca and P per $kg^{0.75}$ (FEDIAF, 2020)

surpassing the RNL were considered undersupplied and nutrients exceeding the nutritional maximum (max) per kg dry matter (DM) (FEDIAF, 2020) or safe upper limit (SUL) per kg DM (NRC, 2006) were considered heavily oversupplied.

After calculating the average daily ration of each individual participant, blood samples were taken. In all animals, 2.5 ml serum and 0.5 ml EDTA plasma were obtained from the front limb at the cephalic vein or the lateral saphenous vein on the hind limb after 12 hours of fasting. This occurred during a visit to the Clinic for Small Animal Internal Medicine of the LMU Munich or the veterinary practice '*Kreuzbergl*' (Klagenfurt, Austria). All samples were prepared in the in-house laboratory and then cooled and protected from light or, in the case of the EDTA plasma, frozen at −18°C and sent for further blood analysis to the accredited laboratory SYNLAB.vet GmbH (Augsburg, Germany). For all transports, special transport devices for blood samples were used to guarantee protection from light as well as a continuous cooling.

The blood screening test '*Barfer-Profil Hund*' offered by SYNLAB.vet GmbH included the levels of total Ca, P, Ca/P ratio, total thyroxine (T4), vitamin A (retinol) and vitamin D (25-hydroxyvitamin D3), I, Cu and Zn. In addition, the blood levels of urea, uric acid, creatinine and taurine were analysed. Analyses performed were as follows: (a) photometry for Ca, P, Cu, Zn, urea, uric acid and creatinine, (b) chemiluminescence immunoassay for vitamin D and T4, (c) high-performance liquid chromatography (HPLC) for Vitamin A, (d) inductively coupled plasma mass spectrometry (ICPMS) for I and (e) ion-exchange chromatography (IEC) for taurine.

The results of the analysed parameters are presented in mean ± standard deviation (SD) of the RMBD group to mean ± SD of the control group and corresponding statistical significance

(p-value). Percentage values refer to the given laboratory reference ranges including lower reference range (LR) and upper reference range (UR) for each parameter.

3 | STATISTICAL METHODS

The distribution of all continuous parameters was assessed using the Shapiro–Wilk normality test. For all normally distributed parameters, a Student's or Welch t test was performed. For normally distributed data, Levene's Test for homogeneity of variance between compared groups was performed. For non-normally distributed parameters Wilcoxon rank-sum test was performed. Due to the mostly non-normally distributed parameters, Kendall method was applied to test the correlation between blood and feed parameters. A Fisher's Exact test regarding the association of categorical parameters was performed between the control group and the RMBD group. Statistical significance was considered at $p \le 0.05$. All statistical analyses were performed using R version 3.6.3 (2020–02–29).

4 | RESULTS

4.1 | Computer-based diet calculations

The average nutrient supply of the RMBD group shows significant differences compared to the control group. Of the RMBD group, 94.0% (78/83) show at least one or more nutritional imbalances of the examined parameters followed by 66.3% (55/83) with five imbalances or more, whereas 57.1% (12/21) of the rations of the control group show no imbalance, 28.6% (6/21) one and 14.3% (3/21) two. The detailed results of the ration evaluation between RMBD group and control group and associated recommended nutrient requirements according to FEDIAF (2020) and NRC (2006) are shown in Tables 1 and 2.

The difference in calcium supply between the RMBD group (one extreme case neglected) and the control group is not significant ($p = 0.108$), whereas the variation in the RMBD group is notably larger with more outliers. In the RMBD group, 19.3% (16/83) are undersupplied with Ca, 12.0% (10/83) even exceed the max for Ca recommended by FEDIAF (2020) and are thus heavily oversupplied. In the control group, no diet is above the nutritional max or below the RNL.

The difference in phosphorus supply between the RMBD and control group is not significant ($p = 0.061$), whereas the variation in the RMBD group is larger with more outliers. In the RMBD group, 10.8% (9/83) are undersupplied with P, and 13.3% (11/83) exceed the max for P according to FEDIAF (2020) and are thus heavily oversupplied. In contrast, the control group shows no ration surpassing the RNL or exceeding the nutritional max.

There is no significant difference in Ca/P ratio of the diet ($p = 0.736$) between RMBD and control group, although all diets of the control dogs show an optimal ratio between 1.3 and 2.0 (NRC, 2006). Accordingly, in the RMBD group, 19.3% (16/83) of the rations show ratios below 1.0, and 8.4% (7/83) are above 2.0.

A significant difference in vitamin A content between RMBD rations and control rations ($p = 0.016$) was detected. In detail, 100% (21/21) of the rations in the control group contain sufficient amounts of vitamin A to meet the RNL, whereas 9.7% (8/83) of the RMBD rations are below the RNL. No ration, either in the RMBD or control group exceeds the nutritional max for vitamin A (FEDIAF, 2020). Furthermore, the variation of vitamin A contents in RMBD rations is more than 4 times higher than in the control group.

The differences in vitamin D contents in the rations of the two groups are highly significant ($p < 0.001$). In percentage terms, 90.5% (19/21) of the control group is supplied with vitamin D covering the requirements for the RNL, in contrast to only 36.1% (30/83) in the RMBD group. In addition, 63.9% (53/83) of the RMBD rations are undersupplied with vitamin D compared to only 9.5% (2/21) of the control group. One ration of the RMBD group clearly exceeds the legal maximum of 2272 IU vitamin D per kg DM feed (Regulation (EU) 2017/1492) being fed high amounts of cod liver oil, even though this limit does not apply, as no feed additive according to Regulation (EU) 1831/2003 was used. On the other hand, it is only slightly beneath the nutritional max of 3200 IU per kg DM according to FEDIAF (2020).

The differences in iodine supply between the RMBD and control group is also highly significant ($p < 0.001$). Only 29.0% (24/83) of the RMBD rations are sufficient to meet the requirements for the RNL, compared to 95.2% (20/21) in the control group. In addition, 60.2% (50/83) of the RMBD are undersupplied, and 10.8% (9/83) exceed the SUL for I according to NRC (2006) and are thus heavily oversupplied, whereas in the control group, 0% (0/21) are undersupplied, and 4.8% (1/21) are also considered heavily oversupplied due to exceeding the SLU according to NRC (2006).

There is a highly significant difference between the copper supply in the RMBD group and the control group ($p < 0.001$). In percentage terms, only 36.1% (30/83) of the RMBD rations meet the requirements for the RNL and 63.9% (53/83) are undersupplied as opposed to 100% (21/21) of the control group meeting the RNL for Cu.

The difference in zinc supply between RMBD and control group is highly significant ($p < 0.001$). Only 24.1% (20/83) of the RMBD rations meet the requirements for the RNL and 75.9% (63/83) are below the RNL compared to 85.7% (18/21) of the control group meeting the requirements for the RNL and 14.3% (3/21) being considered undersupplied.

The difference in crude protein content between RMBD and control group is highly significant ($p < 0.001$). In percentage terms, 97.6% (81/83) of the RMBD rations supply sufficient amounts of protein to cover the RNL, 12.1% (10/83) of which rations provide more than 3 times the RNL according to FEDIAF (2020). In contrast, 90.5% (19/21) of the rations of the control meet the RNL and no ration provides more than 3 times the RNL. In addition, 2.4% (2/83) of the RMBD dogs and 9.5% (2/21) of the control dogs receive less than the RNL for crude protein according to FEDIAF (2020), but not less than the RA according to NRC (2006).

4.2 | Blood levels

Blood was taken from 83 RMBD-fed dogs and 21 commercially fed control dogs after successful calculation of the rations. Regardless of whether the rations were balanced or not, values below, in, and above the laboratory reference range were found. Figure 1 shows the percentage distribution of the results in relation to the reference levels for the control group and the RMBD group. The detailed results of the blood analysis between RMBD group and control group are shown in Table 3.

The mean blood calcium level of both groups is similar without significant differences (2.52 ± 0.15 to 2.51 ± 0.12 mmol/l; $p = 0.952$). The blood levels of 4 RMBD dogs and 1 dog in the control group are below LR, none above UR. Of these dogs, 2 are meeting the nutritional requirements for Ca (FEDIAF, 2020), 1 is heavily undersupplied, and 3 are supplied with the 2.6- to 3.12-fold of the recommended Ca amount.

The mean blood phosphate level of the RMBD group is significantly lower than the mean of the control group (1.16 ± 0.25 to 1.28 ± 0.26 mmol/l; $p = 0.05$). In the RMBD group, 6.0% (5/83) dogs are below LR, whereas none of the control dogs are below LR or above UR. The calculated Ca/P ratio in the blood shows no significant difference between the two groups (2.29 ± 0.62 to 2.05 ± 0.43; $p = 0.072$).

The mean blood iodine level of both groups is similar without significant difference (1.32 ± 1.20 to 1.11 ± 0.45 μmol/l; $p = 0.688$). In the RMBD group, 12.2% (10/82) are below LR and 25.6% (21/82) above UR, in contrast to 4.8% (1/21) below LR and 14.3% (3/21) above the UR in the control group.

The mean blood urea level of the RMBD group is similar to the mean level of the control group and without significant difference (6.69 ± 1.86 to 6.47 ± 2.04 mmol/l; $p = 0.512$). In the RMBD group, 18.1% (15/83) are above UR. For the control group, the corresponding result is 19.1% (4/21) above UR.

No dog in both groups is above UR (<60 μmol/l) for uric acid.

The mean blood retinol level of the RMBD group is significantly lower than the mean of the control group (2.92 ± 1.16 to 3.7 ± 1.14 μmol/l; $p = 0.012$). This is shown by the fact that 56.6% (47/83) of the blood levels of RMBD dogs are below LR. Also, these are in or below the range of the lowest 25% of the control animals. In the control group, 28.6% (6/21) are below LR.

The mean blood 25-hydroxyvitamin D3 level of the RMBD group compared to the mean blood level of the control group is without a significant difference (354.48 ± 375.38 nmol/l to 222.7 ± 76.26 nmol/l; $p = 0.315$). In detail, in the RMBD group, 15.7% (13/83) are very far above UR with a maximum value of 2030 nmol/l (reference range 50–365 nmol/l). In the control group, however, only one dog is slightly above UR.

The mean blood copper level of the RMBD group is significantly lower than the mean of the control group (8.48 ± 3.41 to 14.9 ± 8.86 μmol/l; $p < 0.001$). In addition, 22.0% (18/82) of RMBD dogs are below LR compared to 9.5% (2/21) in the control group. However, only 2.4% (2/82) of RMBD dogs are above UR compared to 33.3% (7/21) in the control group.

The mean blood zinc level of the RMBD group is significantly lower than the mean of the control group (9.62 ± 2.46 to 11.42 ± 2.13 μmol/l; $p = 0.001$). Furthermore, 4.9% (4/82) of RMBD dogs are below LR, whereas none of the control dogs are below LR or above UR.

The mean blood creatinine level of the RMBD group to the mean blood level of the control group is without a significant difference (78.85 ± 20.16 μmol/l to 83.65 ± 14.75 μmol/l; $p = 0.177$). Only 1 dog (1.2%) in the RMBD group is above UR (>150 μmol/l).

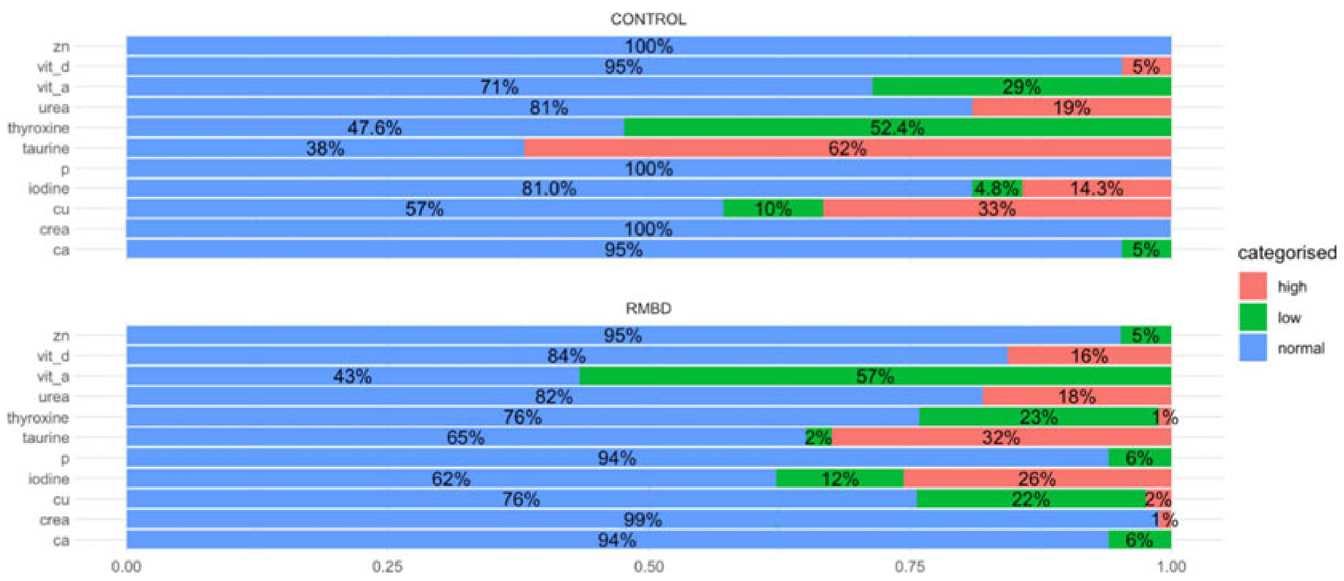

FIGURE 1 Percentage distribution of values measured in blood between the control group and the raw meat-based diet (RMBD) group into normal, below and above the reference range of SYNLAB.vet GmbH internal laboratory reference values (2020). Note: normal, within reference or below tolerance; zn, zinc; p, phosphate; i, iodine; cu, copper; crea, creatinine; ca, calcium

TABLE 3 Levels (mean ± standard derivation (minimum–maximum)) of nutrients analyzed in serum and plasma of the raw meat-based diet (RMBD) group and the control group and their respective reference levels according SYNLAB.vet GmbH, 2020

	Mean blood values				
	RMBD (n = 83)	Control group (n = 21)	*p*-value	Reference level	
Creatinine	78.85 ± 20.16 (42.40–177.00)	83.65 ± 14.75 (61.00–117.60)	0.177	0–150	µmol/l
Urea	6.69 ± 1.86 (3.51–14.32)	6.47 ± 2.04 (4.00–11.82)	0.512	3.2–8.2	mmol/l
Uric acide	59.00 ± 0.00 (<59)	59.00 ± 0.00 (<59)	n/a	0–60	µmol/l
Calcium	2.52 ± 0.15 (1.88–2.94)	2.51 ± 0.12 (2.25–2.71)	0.952	2.3–3.0	mmol/l
Phosphate	1.16 ± 0.25 (0.39–1.89)	1.28 ± 0.26 (0.87–1.75)	**0.050**	0.82–2.0	mmol/l
Ca/P ratio	2.29 ± 0.62 (1.40–6.10)	2.05 ± 0.43 (1.40–2.90)	0.072	n/a	
Taurine	108.24 ± 40.78 (38.30–231.00)	142.56 ± 54.55 (54.80–249.00)	**0.008**	46–116	µmol/l
Thyroxine	26.53 ± 10.97 (2.26–72.59)	19.79 ± 8.25 (7.73–36.14)	**0.007**	19.3–57.9	nmol/l
Iodine	1.32 ± 1.20 (0.15–6.66)	1.11 ± 0.45 (0.30–2.14)	0.688	0.4–1.6	µmol/l
Zinc	9.62 ± 2.46 (4.59–16.83)	11.42 ± 2.13 (8.26–17.90)	**0.001**	5.4–23.0	µmol/l
Copper	8.48 ± 3.41 (3.14–27.00)	14.9 ± 8.86 (6.28–33.13)	**<0.001**	6.9–18.7	µmol/l
25-Hydroxy-D3	354.48 ± 375.38 (57.50–2030.00)	222.7 ± 76.26 (105.00–450.00)	0.315	50–365	nmol/l
Retinol	2.92 ± 1.16 (0.56–6.66)	3.7 ± 1.44 (2.34–7.90)	**0.012**	2.9–10.7	µmol/l

The mean plasma taurine level of the RMBD group is significantly lower than the mean of the control group (108.24 ± 40.78 to 142.56 ± 54.55 µmol/l; p = 0.008). In the RMBD group, 2.5% (2/79) of the dogs are below LR, 30.4% (24/79) of the RMBD dogs and 61.9% (13/21) of the control group are above UR.

The mean blood thyroxine level of the RMBD group is significantly higher than the mean of the control group (26.53 ± 10.97 nmol/ to 19.79 ± 8.25 nmol/l; p = 0.007). It is 22.9% (19/83) of the RMBD group to 52.4% (11/21) of the control group below LR. One dog (1.2%) in the RMBD group is well above the UR (72.49 nmol/l; UR = 57.9 nmol/l).

The calculated parameters from the rations and their corresponding results in the blood show a low to very low negative correlation with −0.031 for creatinine/crude protein, −0.08 for Ca/P and −0.16 for vitamin A, whereby only vitamin A shows a significance of p = 0.05. A positive correlation can be recorded for Ca with 0.11, P with 0.16, I with 0.16, vitamin D with 0.21, Zn with 0.14, Cu with 0.024, and P/crude protein with 0.079. Significant with p = 0.05 are the correlations between ration and blood for Zn, P and iodine. Also significant with p = 0.01 is vitamin D. For all other parameters, no significance could be detected.

5 | DISCUSSION

In our study, not one of the investigated parameters could show a correlation above 0.5 with $p < 0.05$. Similar to another study (Shakhar et al., 2010) this further supported our main hypothesis that there is little to no correlation between the level of supply and the results of the '*Barfer-Profil Hund*' (SYNLAB.vet, 2020), contrary to another study that only controlled the type of feeding, but not the exact composition thereof (Frisk, 2019). This may be related to the fact that a diet-related inadequate supply of nutrients and the resulting clinical symptoms can (De Fornel-Thibaud et al., 2007), but not necessarily must, lead to altered blood parameters (Becker et al., 2012; Wichert et al., 2002). Correspondingly, in our study, 62% of the RMBD dogs showed a normal I value and 26% an increased I value in serum, although only about 29% of the dogs were supplied with I more than the RNL, and even 60% below the RNL (FEDIAF, 2020). The lack of correlation here could also be due to the adaptation of the organism to fluctuating I-contents, sometimes lasting one year (Fritz, 2018; Zentek, 2016b). The reasons are, for example, strong fluctuations in different batches of seaweed meal (Kölle & Schmidt, 2015), or sudden change from commercial feed to a RMBD without I supplementation, or the feeding of iodine-containing fish only once a week, which was already classified as insufficient by Dillitzer et al., (2012). Also, the nutrition consultation at the Clinic for Small Animal Internal Medicine of the LMU Munich was able to observe empirically that clinical symptoms developed between 12 and 24 months after the malnutrition started (Kölle & Schmidt, 2015). Another possibility would be to determine iodine in the urine (Lange, 2017; Rasmussen et al., 1999), as it reflects more accurately the alimentary iodine supply (Fritz, 2018). Therefore, apparently normal blood values despite inadequate nutritional supply weighs owners in a false sense of security. This is well demonstrated by the very tight Ca homeostasis (Ettinger et al., 2017; Fritz, 2018; Hand, 2002; NRC, 2005; Zentek, 2016c) as results show imbalances in 31.3% of RMBD but normal serum Ca in 93% of the RMBD group. It also applies to other parameters such as vitamin A, for which a change in blood levels only becomes visible after the liver stores are depleted. Prior to this, only a liver biopsy can reflect the actual supply level (Barko & Williams, 2018; Fritz, 2018; Tran et al., 2007; Zentek, 2016d). This is also apparent in our results. In the RMBD group, 94.0% (78/83) of the rations have at least one, 66.3% have even five or more nutritional imbalances, although this does not have a significant effect on blood values, which could also be shown in horses (Wichert et al., 2002).

About 13.3% of RMBD dogs receive more P than the nutritional max according to FEDIAF. This overloaded P must be excreted via the kidneys (Chang & Anderson, 2017; Zentek, 2016ca), which may lead to a significant renal burden, especially in the case of P excesses (Dobenecker & Siedler, 2016). It is suspected that this burden can lead to chronic kidney disease (CKD) (Dobenecker, 2019; Dobenecker & Siedler, 2016). Regarding this, Böswald et al. were able to show that 46% of the investigated dogs with CKD were fed more than 150% of the RA (NRC, 2006) for P (Böswald et al., 2018) although the main problem being inorganic P sources usually found in dry feed or supplements. Other studies also further solidify the suspicion of negative effects of certain P sources and/or P excess on renal health (Chang & Anderson, 2017; Dobenecker, 2018, 2019; Dobenecker & Siedler, 2016, 2017; Herbst & Dobenecker, 2019). In our study, approximately 65.0% (54/83) of RMBD dogs and 90.5% (19/21) of the control group receive more than 1.5 times the RA according to the NRC. Despite the partially massive oversupply, no dog is above the UR as mainly organic sources of P can be found in RMBD but 6.0% of RMBD dogs are below the LR. Common reasons for hypophosphatemia would be diabetic ketoacidosis, tumour-associated hypercalcemia or primary hyperparathyroidism (Ettinger et al., 2017; Nelson & Couto, 2019), which with current knowledge does not apply to any of the study participants. Other more uncommon reasons include (a) decreased alimentary intake, (b) vitamin D deficiency, (c) renal disease, (d) iatrogenic causes such as phosphate binders or (e) respiratory and metabolic acidosis (Ettinger et al., 2017; Nelson & Couto, 2019). Points (d) and (e) are unlikely because it would require an already established renal problem or underlying disease that would have been an exclusion criteria. Point (c), the possibility of masked early kidney disease, especially in RMBD dogs, may be a factor. Vitamin D deficiency also seems to be a plausible cause, as 63.9% of RMBD dogs are considered undersupplied according to the RNL. Point (a) may be a cause as 10.8% of RMBD dogs get less than the RNL for P according to FEDIAF, in addition to the fact that the frequent inappropriate Ca/P ratios may further decrease P absorption. Another factor that could contribute to incorrect high P serum values is haemolysis (Carlson, 1989; DiBartola, 2011). Low-grade haemolysis was detected in 11 of the 104 study participants. Furthermore, even though the Ca/P ratio between RMBD and control dogs show no significant difference, it should be accounted for, that 27.7% of RMBD provide Ca/P ratios beneath, or above recommendations (FEDIAF, 2020), in contrast to 0% in control dogs, as previously shown by Pedrinelli et al., (2019) and Vecchiato and Dobenecker (2018). Strong deviations from the ideal ratio can lead to severe health problems, especially in growing dogs (Becker et al., 2012; De Fornel-Thibaud et al., 2007; Dobenecker, 2018).

Although about 64% of RMBD dogs are undersupplied with vitamin D according to the RNL, about 86.8% are within and 13.3% above the reference for 25-hydroxyvitamin D3 (25(OH)D3) in serum. Possible explanations could be (a) highly varying vitamin D contents in fish (Jakobsen et al., 2019; Mattila et al., 1995; Schmid & Walther, 2013; Selting et al., 2016), (b) ready-made, frozen packages with varying contents of raw frozen fish, as also present in our study, (c) low correlation between oral intake of vitamin D and 25(OH)D3 in blood as already shown in other studies (Weidner & Verbrugghe, 2017; Young & Backus, 2016), (d) 25(OH)D3 acting as a negative acute phase reagent (Waldron et al., 2013), (e) the emerging hypothesis, also shown in human medicine, that 25(OH)D3 is not suitable to evaluate vitamin D status due to low levels in chronic inflammation (Mangin et al., 2014) and, (f) high variability of 25(OH)D3 in healthy or RMBD-fed dogs (Selting et al., 2016; Sharp et al., 2015; Young & Backus, 2016). Finally, it is important to point out possible breed differences, especially between small and large dogs and thus possible different metabolization of 25(OH)D3 (Hazewinkel & Tryfonidou, 2002). Taken all these factors into account, the measurement of 25(OH)D3 for the stated purpose in a RMBD blood profile seems to be of no use.

Thyroxine showed a strong significant difference between the two groups with the highest and lowest measured values in the RMBD group. These values may relate to (a) possible residues of thyroid glands in the meat fed (Köhler et al., 2012) as 1 dog with T4 values above the UR was diagnosed with hyperthyroidism posterior to participating in this study, that normalized after a change of the diet, (b) excessive iodine intake, as seen in the results, can lead to different T4 values in blood (Markou et al., 2001), (c) breed-related factors in, for example sight hounds and Nordic breeds, leading to typical lower T4 values (Shiel et al., 2007), although dogs of this breeds that participated in our study (only in the RMBD group) showed unusually high values within the range, (d) negative correlation of body weight and T4 blood values (Nelson & Couto, 2019) as there are very small dogs (Chihuahua) to very heavy dogs (Bernese Mountain Dog) to a more homogenous control group and (e) the overlapping range that healthy dogs and those with hypothyroidism share in T4 analysis (Nelson & Couto, 2019). In conclusion, for the sole purpose of a pre-screening, T4 may be useful if the mentioned limiting factors are taken into account, and if a trend can be seen after regular checkups.

Zn is one of the frequently undersupplied nutrients in home-made RMBD rations (Dillitzer et al., 2011; Pedrinelli et al., 2019; Streiff et al., 2002; Vervuert & Rückert, 2017), which also applies to our study (75.9% of the RMBD below RNL). The poor correlation of the nutritional supply with the blood level and possible influence on it by, for example, disease is already described by other studies and authors (Huber et al., 1991; Kamphues, 2014; Logas et al., 1993) which concludes that normal values cannot rule out an excess or deficient supply. Fieten et al., (2013) described a possible alternative by measuring the basal value of Zn in urine, but further studies are still needed.

Cu deficiency is also common in home-made RMBD (Dillitzer et al., 2011; Stockman et al., 2013; Streiff et al., 2002; Weeth, 2013). This was confirmed in the current study, as about 63.9% of RMBD dogs showed insufficient nutritional supply according to RNL. Nevertheless, no significant correlation between nutritional supply and serum levels could be found, suggesting that the Cu serum level does not provide any information about the liver Cu status, as already mentioned by Fieten et al., (2012). Therefore, a clear evaluation of the Cu status can only be made by liver biopsy (Kölle &

Schmidt, 2015; Zentek, 2016d), which is clearly unrelated to the level of effort. As for Zn, Fieten et al., (2013) described the possibility of Cu measurement via urine following further studies.

Only two of the dogs examined had a plasma taurine level below LR of 46 nmol/l. Both belonged to the RMBD group, whereby one of the dogs (German Shepherd) received a normal RMBD with grains, as did the partner dog (Beauceron) that showed a normal taurine plasma value. The other dog with low plasma taurine was fed exotic meats without cereals, together with lamb and rice, which is all suspected to promote taurine deficiency (Fascetti et al., 2003; Freeman et al., 2018; Johnson et al., 1998). On the other hand, two dogs (Doberman and Greyhound) belonged to the RMBD group with normal plasma taurine levels but suspected or confirmed dilated cardiomyopathy (DCM). In this case, one dog received a grain-free diet with buckwheat, which has been associated with hepatitis in dogs (Lineva et al., 2019), as well as the partner animal without suspected DCM and normal blood values. The second dog received a grain-free diet with exotic protein sources, lamb and rice. Due to the fact that a high plasma taurine content cannot completely rule out DCM (Kramer et al., 1995), as there is probably also DCM without relation to food but, for example, to the breed (Freeman et al., 2018), normal taurine plasma levels should also be considered with care. Nevertheless, it is recommended in cases of suspected DCM to measure plasma and whole blood taurine (Ettinger et al., 2017; Freeman et al., 2018) and always include the complete dietary history (Kaplan et al., 2018). Therefore, plasma taurine is only of limited use for the stated purpose of a RMBD blood profile.

The overall weak significance, especially considering the lack of an assessment of a medium to longer-term supply of nutrients, gives rise to doubts that a RMBD blood profile can have the same relevance as a professional ration check. In contrast, a computer-aided ration calculation can show nutritional imbalances at any given time and thus contribute to corrections (Kölle & Schmidt, 2015; Vervuert & Rückert, 2017). The nutrition consultation at the Clinic for Small Animal Internal Medicine of the LMU Munich observed empirically poor kidney health in some dogs fed RMBD, especially older ones. This may be related to the high amounts of protein and therefore P in the diet, which can be an additional burden, especially for mature organisms. In this context, the usefulness of the additional parameters urea, uric acid and creatinine to assess early-stage kidney problems seems to be limited, as a single measurement will not show beginning problems via an increase of blood values within the reference range. These can only be detected in the trend profile, for which repeated or regular measurements would be necessary (Ettinger et al., 2017; Nelson & Couto, 2019). Also, measuring symmetric dimethylarginine (SDMA) would be a good possibility to monitor early-stage kidney disease (Kopke et al., 2018; Nabity et al., 2015). However, a benefit, which should not be ignored, is shown in the willingness of some dog owners (30% in the present study) to approach a nutrition consultation after any deviations from the reference in blood analysis in order to have a ration optimization carried out.

6 | CONCLUSION

The benefit of a blood profile for dogs fed RMBDs should be the examination of the general nutrient supply situation or an early indication of a deficiency in RMBD feeding, as well as an indication of nutritive hyperthyroidism due to feeding thyroid-containing tissue (SYNLAB.vet, 2020). Due to the fact that a blood analysis is always a momentary impression and that there are clear limitations in the above-mentioned application due to special physiology and homeostasis of the individual parameters, the general statement that a RMBD blood profile represents the general nutritional supply cannot be accurate.

The calculated imbalances in RMBD feeding are, for the most part, not significantly reflected in the RMBD blood profile and for most parameters there is, therefore, no direct correlation between feeding and blood levels. Although it should be considered, that in the mean a RMBD was fed for 2.9 ± 2.1 years (min 0.5 years; max 10.0 years) and body storages could be depleted more or less. Based on these results, a mere examination of the nutrient supply by means of blood analysis does not seem to be sufficient enough, even if the sample size is comparatively small. Specially selected parameters and examination intervals can provide useful information on nutrition and pathological processes but cannot replace computer-aided ration calculation. Furthermore, it was again shown that there is still much potential for improvement in the composition of a RMBD and that owners need to be educated about the possible advantages and disadvantages, unsuitable feed and risks of a self-created RMBD.

7 | ANIMAL WELFARE STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes. The protocol is approved under number 127–10–06–2018. All participants in the study were selected because their owners particularly wanted their dogs to be checked by a commercially available blood profile for RMBD-fed dogs.

ACKNOWLEDGMENTS

We thank the team of the veterinary practice '*Kreuzbergl*' and especially Mag. Daniela Bezerédj-Babarczy for their active support in collecting samples as well as the staff of the Clinic for Small Animal Internal Medicine, Ludwig-Maximilians University Munich. We also want to thank SYNLAB.vet GmbH, Augsburg, Germany for carrying out the blood analyses and for financial support of the present study. Open Access funding enabled and organized by Projekt DEAL. WOA Institution: LUDWIG-MAXIMILIANS-UNIVERSITAET MUNCHEN Blended DEAL: Projekt DEAL

CONFLICT OF INTERESTS

All blood analyses were carried out and paid by SYNLAB.vet GmbH. SYNLAB.vet GmbH did not participate in this study and the results were in no way influenced by the laboratory.

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How to cite this article: Hajek, V., Zablotski, Y., & Kölle, P. (2022). Computer-aided ration calculation (Diet Check Munich©) versus blood profile in raw fed privately owned dogs. *Journal of Animal Physiology and Animal Nutrition*, 106, 345–354. https://doi.org/10.1111/jpn.13601